TOXICOLOGICAL PROFILE FOR ACETONE

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

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UPDATE STATEMENT

This edition supersedes any previously released draft or final profile.

Toxicological profiles are revised and republished as necessary, but no less than once every three years. For information regarding the update status of previously released profiles contact ATSDR at:

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FOREWORD

The Superfund Amendments and Reauthorization Act (SARA) of 1986 (Public Law 99-499) extended and amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). This public law directed the Agency for Toxic Substances and Disease Registry (ATSDR) to prepare toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List and that pose the most significant potential threat to human health, as determined by ATSDR and the Environmental Protection Agency (EPA). The revised list of the 275 most hazardous substances was published in the Federal Register on October 28, 1992 (57 FR 48801). For prior versions of the list of substances, see Federal Register notices dated April 17, 1987 (52 FR 12866); October 20, 1988 (53 FR 41280); October 26, 1989 (54 FR 43619); October 17, 1990 (55 FR 42067); and October 17, 1991 (56 FR 52166).

Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list. Each profile must include the following:

(A) The examination, summary, and interpretation of available toxicological information and epidemiological evaluations on a hazardous substance in order to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects.

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure that present a significant risk to human health of acute, subacute, and chronic health effects.

(C) Where appropriate, identification of toxicological testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

This toxicological profile is prepared in accordance with guidelines developed by ATSDR and EPA. The original guidelines were published in the Federal Register on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile is intended to succinctly characterize the toxicological and adverse health effects information for the hazardous substance being described. Each profile identifies and reviews the key literature (that has been peer-reviewed) that describes a hazardous substance's toxicological properties. Other pertinent literature is also presented, but described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

Each toxicological profile begins with a public health statement, that describes in nontechnical language, a substance's relevant toxicological properties. Following the public health statement is information concerning levels of significant human exposure and, where known, significant health effects. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to protect public health will be identified by ATSDR and EPA. The focus of the profiles is on health and toxicological information; therefore, we have included this information in the beginning of the document.

Foreword

The principal audiences for the toxicological profiles are health professionals at the federal, state, and local levels, interested private sector organizations and groups, and members of the public.

This profile reflects our assessment of all relevant toxicological testing and information that has been peer reviewed. It has been reviewed by scientists from ATSDR, the Centers for Disease Control and Prevention (CDC), and other federal agencies. It has also been reviewed by a panel of nongovernment peer reviewers and was made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

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THE PROFILE HAS UNDERGONE THE FOLLOWING ATSDR INTERNAL REVIEWS:

1. Green Border Review. Green Border review assures the consistency with ATSDR policy.

2. Health Effects Review. The Health Effects Review Committee examines the health effects chapter of each profile for consistency and accuracy in interpreting health effects and classifying endpoints.

3. Minimal Risk Level Review. The Minimal Risk Level Workgroup considers issues relevant to substance-specific minimal risk levels (MRLs), reviews the health effects database of each profile, and makes recommendations for derivation of MRLs.

4. Quality Assurance Review. The Quality Assurance Branch assures that consistency across profiles is maintained, identifies any significant problems in format or content, and establishes that Guidance has been followed.

PEER REVIEW

A peer review panel was assembled for acetone. The panel consisted of the following members:

1 . Dr. Elizabeth Jeffery, Associate Professor of Toxicology, University of Illinois, Urbana, Illinois

2 . Dr. Peter G. Lacouture, Associate Director, Clinical Research, Purdue Frederick Company, Norwalk, Connecticut

3 . Dr. John Morris, Associate Professor of Pharmacology and Toxicology, Toxicology Program, School of Pharmacy, University of Connecticut, Storrs, Connecticut.

These experts collectively have knowledge of acetone's physical and chemical properties, toxicokinetics, key health end points, mechanisms of action, human and animal exposure, and quantification of risk to humans. All reviewers were selected in conformity with the conditions for peer review specified in Section 104(i)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

Scientists from the Agency for Toxic Substances and Disease Registry (ATSDR) have reviewed the peer reviewers' comments and determined which comments will be included in the profile. A listing of the peer reviewers' comments not incorporated in the profile, with a brief explanation of the rationale for their exclusion, exists as part of the administrative record for this compound. A list of databases reviewed and a list of unpublished documents cited are also included in the administrative record.

The citation of the peer review panel should not be understood to imply its approval of the profile's final content. The responsibility for the content of this profile lies with the ATSDR.

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1. PUBLIC HEALTH STATEMENT

This public health statement tells you about acetone and the effects of exposure. This information is important because this chemical may harm you.

The Environmental Protection Agency (EPA) has identified 1,350 hazardous waste sites as the most serious in the nation. These sites make up the National Priorities List (NPL) and are targeted for long-term federal clean-up. Acetone has been found in at least 560 NPL sites. However, it's unknown how many NPL sites have been evaluated for this substance.

As EPA tests more sites, the sites with acetone may increase. This is important because exposure to acetone may harm you and because these sites are or may be sources of exposure.

When a large industrial plant or a small container releases a substance, it enters the environment. This release does not always lead to exposure. You are exposed to a substance only when you come in contact with it by breathing, eating, touching, or drinking.

If you are exposed to acetone, many factors determine if you'll be harmed and how badly. These factors include the dose (how much), the duration (how long), and how you're exposed. You must also consider the other chemicals you're exposed to and your age, sex, nutritional status, family traits, lifestyle, and state of health.

1.1 WHAT IS ACETONE?

Acetone is a chemical that is found naturally in the environment and is also produced by industries. Low levels of acetone are normally present in the body from the breakdown of fat; the body can use it in normal processes that make sugar and fat (see Section 1.4). Acetone is a colorless liquid with a distinct smell and taste. People begin to smell acetone in air at 100 to 140 parts of acetone in a million parts of air (ppm), though some can smell it at much lower levels. Most people begin to detect the presence of acetone in water at 20 ppm. Acetone evaporates readily into the air and mixes well with water. Most acetone produced is

used to make other chemicals that make plastics, fibers, and drugs. Acetone is also used to dissolve other substances. You will find further information on the physical and chemical properties of acetone, and its production and use in Chapters 3 and 4.

1.2 WHAT HAPPENS TO ACETONE WHEN IT ENTERS THE ENVIRONMENT?

Acetone enters the air, water, and soil as a result of natural processes and human activities. Acetone occurs naturally in plants, trees, volcanic gases, and forest fires. People and animals breathe out acetone produced from the natural breakdown of body fat. Acetone is also released during its manufacture and use, in exhaust from automobiles, and from tobacco smoke, landfills, and certain kinds of burning waste materials. The levels of acetone in soil increase mainly because of acetone-containing wastes being buried in landfills. Acetone is present as a gas in air. Some acetone in air is lost when it reacts with sunlight and other chemicals. Rain and snow also remove small amounts of acetone from the atmosphere and, in the process, deposit it on land and water. About half the acetone in a typical atmosphere at any time will be lost in 22 days. Microbes (minute life forms) in water remove some acetone from water. Some acetone in water will evaporate into air. About half the acetone in a stream will be removed from water in less than a day. Fish do not store acetone from water in their bodies. Microbes in soil remove part of the acetone in soil. Some is lost from soil by evaporation. Acetone molecules do not bind tightly to soil. Rainwater and melted snow dissolve acetone and carry it deeper into the soil to groundwater. You will find further information about the fate and movement of acetone in the environment in Chapter 5.

1.3 HOW MIGHT I BE EXPOSED TO ACETONE?

Your body makes small amounts of acetone. You can be exposed to a small amount of acetone by breathing air, drinking water, and eating food with acetone. You can also be exposed by contact with household chemicals with acetone. Several consumer products, including certain nail polish removers, particle board, some paint removers, many liquid or paste waxes or polishes, and certain detergents or cleansers, contain acetone. You can also be exposed to acetone if you are exposed to isopropyl alcohol, because isopropyl alcohol changes

to acetone in the body. The level of acetone in air and water is generally low. The amount of acetone in the air of cities is generally higher than in remote and rural areas. The typical level of acetone in the air of cities in the United States is about 7 parts of acetone per billion parts of air (ppb). The level of acetone in air inside homes is usually slightly higher than in outside air (8 ppb versus 7 ppb). This is because of household chemical use inside homes. Acetone in drinking water is so low that its levels have not been measured in many samples. In a national survey, the acetone level in drinking water from Seattle, Washington, was 1 ppb. Acetone occurs naturally in many fruits and vegetables. The amount of acetone in food does not increase because of processing or packaging. The average amount of acetone an adult in the United States gets from food is not known.

People who work in certain industries that process and use acetone can be exposed to higher levels than the general populace. These industries include certain paint, plastic, artificial fiber, and shoe factories. Professional painters and commercial and household cleaners are also likely to breathe or touch higher acetone concentrations than the general population. As a member of the general public, you may be exposed to higher than normal levels of acetone if you smoke cigarettes, frequently use acetone nail polish removers, live near landfill sites that contain acetone, live near busy roadways (because automobile exhaust contains acetone), or live near other facilities that are known to release acetone, such as incinerators. The exposure from these sources will be mainly from breathing air that contains acetone or by direct skin contact with it. In addition, children can be exposed to acetone by eating dirt or by placing dirty hands in their mouths after exposing their skin to dirt from landfill sites. You will find further information about acetone exposure in Chapter 5.

1.4 HOW CAN ACETONE ENTER AND LEAVE MY BODY?

Your body normally contains some acetone because it’s made during the breakdown of fat. Your body will make more acetone from body fat if you are on a low-fat diet. In addition to the acetone that your body makes from normal processes, acetone can enter your body if you breathe air that contains acetone, drink water or eat food that contains acetone, or if you touch liquid acetone or soil that contains acetone.

The bloodstream absorbs acetone rapidly and completely from the lungs and stomach. The bloodstream can also absorb acetone from the skin, but less rapidly than from the lungs and stomach. Blood carries acetone to all body organs, but it does not stay there very long.

The liver breaks down acetone to chemicals that are not harmful. The body uses these chemical to make glucose (sugar) and fats that make energy for normal body functions. The breakdown of sugar for energy makes carbon dioxide that leaves your body in the air you breathe out. These are normal processes in the body.

Not all the acetone that enters your body from outside sources is broken down. The amount that is not broken down leaves your body mostly in the air that you breathe out. You also breathe out more carbon dioxide than normal if you are exposed to acetone from sources outside the body because more carbon dioxide is made from the extra acetone.

Only a small amount of acetone that is not broken down leaves the body in the urine. The acetone that is not used to make sugar leaves your body within a few days in the air you breathe out and in the urine. The amount of acetone that enters and leaves your body depends on how much you’re exposed to and for how long. The higher the level of acetone and the longer that you are exposed will cause acetone to leave your body more slowly, but almost all the acetone will leave your body within 3 days after your exposure stops. If you exercise or work while exposed to acetone in air, more will enter your lungs because you breathe faster and more deeply during exercise. For more information on how acetone enters and leaves the body, see Chapter 2.

1.5 HOW CAN ACETONE AFFECT MY HEALTH?

As mentioned in Section 1.4, low levels of acetone are normally present in the body from the breakdown of fat. The body uses acetone in normal processes that make sugar and fats that make energy for normal body functions. Many conditions can lead to higher-than-average amounts of acetone in the body. For example, babies, pregnant women, diabetics, and people who exercise, diet, have physical trauma, or drink alcohol can have higher amounts of acetone

in their bodies. These higher amounts of acetone usually don't cause health problems. In addition, acetone can prevent convulsions.

Most of the information on how acetone affects human health comes from medical exams of workers on a single workday; from lab experiments in humans exposed to acetone in air for a few days; and from cases of people who swallowed acetone-based glue or fingernail polish remover.

Workers and people exposed to acetone in the lab complained that acetone irritated their noses, throats, lungs, and eyes. Some people feel this irritation at levels of 100 ppm acetone in the air, and more people feel the irritation as the level in air increases. The workers who complained of irritation were exposed to levels of 900 ppm or more. Workers exposed to acetone at 12,000 ppm or higher also complained of headache, lightheadedness, dizziness, unsteadiness, and confusion depending on how long they were exposed (from 2 minutes to 4 hours). Two workers exposed for 4 hours became unconscious.

In addition, some people who had casts applied with acetone were exposed to acetone that evaporated into air during and after the casts were applied. These patients became nauseous, vomited blood, and became unconscious. These cases happened many years ago; modern hospitals have different methods that don't use acetone when casts are applied. Some people exposed to acetone in the air at about 250 ppm for several hours in the lab had headaches and lacked energy, and they also had some mild behavioral effects. These effects showed up in tests of how long it takes to react to a visual stimulus or the ability to hear different sounds. Some people exposed to 500 ppm in the air for several hours in the lab had effects on the blood, but other studies showed no effects on the blood at even higher exposure levels.

Some women exposed to 1,000 ppm for about 8 hours in a lab said that their periods came earlier than expected. Workers are not usually exposed to levels higher than 750 ppm anymore because of current government regulations. The regulation says workroom air should contain no more than an average of 750 ppm. Most people can smell acetone in the air at 100 to 140 ppm; that means you will probably smell acetone before you feel effects like

headache and confusion. Levels of acetone in air in rural areas and in cities (less than 8 ppb) are generally lower than this.

People who swallowed acetone or substances that contained acetone became unconscious, but they recovered in the hospital. The amount of acetone that these people swallowed was not always known, but one man swallowed about 2,250 milligrams of pure acetone per kilogram of body weight (2,250 mg/kg). In addition to becoming unconscious, he had tissue damage in his mouth and he later developed a limp, which eventually cleared up, and symptoms similar to diabetes (excessive thirst, frequent urination). The amount of acetone in water or food would never be high enough to cause these effects, but people, especially children, could accidentally swallow enough acetone in nail polish remover or some household cleaners to cause such effects.

In a lab experiment, people who had liquid acetone applied directly on their skin and held there for a half hour developed skin irritation. When the skin was looked at under a microscope, some of the skin cells were damaged.

Animals briefly exposed to high levels of acetone in the air also had lung irritation and became unconscious; some died. Exposure at lower levels for short periods also affected their behavior. Pregnant animals that were exposed to high levels of acetone in air had livers that weighed more than usual and had fewer fetuses. The fetuses weighed less than normal and had delayed bone development. We do not know how exposure to acetone in air for longer than 2 weeks affects animals.

Animals given large amounts of acetone to swallow or drink for short periods had bone marrow hypoplasia (fewer new cells being made), degeneration of kidneys, heavier than normal livers and bigger liver cells, and collapse and listlessness. Pregnant mice that swallowed acetone had lower body weights and produced fewer newborn mice. More of the newborns of mice that had swallowed acetone died than newborns of mice that were not given acetone.

Male rats that swallowed or drank even small amounts of acetone for long periods had anemia and kidney disease. The female rats did not have anemia, but they had kidney disease when they swallowed a much larger amount of acetone than the male rats swallowed. The female rats had livers and kidneys that weighed more than normal, and so did the male rats, but only when they swallowed larger amounts of acetone than the females swallowed. The male rats also had abnormal sperm. The female rats did not have any effects in their reproductive organs. Rats also had signs that acetone caused effects on their nervous systems.

Acetone is irritating to the skin of animals when it is placed directly on their skin, and it burns their eyes when placed directly in their eyes. One kind of animal (guinea pigs) even developed cataracts in their eyes when acetone was placed on their skin.

We do not know whether many of the effects seen in animals would occur in humans. People exposed to acetone were not examined for some effects or could not be examined for effects that can be seen only by looking at internal organs under a microscope. The findings in animals show that male rats are more likely than female rats to get blood and kidney disease and effects on reproductive organs after exposure to acetone. This suggests that men might be more likely to have effects of exposure to acetone than women.

One effect of acetone seen in animals is an increase in the amount of certain enzymes (chemicals in the body that help break down natural substances in the body and chemicals that enter the body). The increase in these enzymes caused by acetone exposure can make some chemicals more harmful. This is one reason that people should be concerned about being exposed to acetone; exposure is very likely to mixtures of chemicals in the environment, near hazardous waste sites, or in the workplace is very likely.

Acetone does not cause skin cancer in animals when it is applied to their skin. We don’t know whether acetone would cause cancer after breathing or swallowing it for long periods, because no tests have been done. The Department of Health and Human Services and the International Agency for Research on Cancer have not classified acetone for carcinogenic

effects. The EPA has determined that acetone is not classifiable as to its human carcinogenicity.

1.6 IS THERE A MEDICAL TEST TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO ACETONE?

Acetone can be measured in the air you breathe out, in the blood, and in the urine. Methods for measuring acetone in breath, blood, and urine are available at most modern testing labs. Doctors' offices may not have the necessary equipment, but your doctor can take blood and urine samples and send them to a testing lab. The measurement of acetone in breath, blood, and urine can determine whether you have been exposed to acetone if the levels are higher than those normally seen. They can even predict how much acetone you were exposed to. However, normal levels of acetone in breath, blood, and urine can vary widely depending on many factors, such as infancy, pregnancy, lactation, diabetes, physical exercise, dieting, physical trauma, and alcohol. The odor of acetone on your breath can alert a doctor that you have been exposed to acetone. An odor of acetone on your breath could also mean that you have diabetes. Because acetone leaves your body within a few days after exposure, these tests can tell only that you have been exposed to acetone within the last 2 or 3 days. These tests cannot tell whether you will experience any health effects related to your exposure.

1.7 WHAT RECOMMENDATIONS HAS THE FEDERAL GOVERNMENT MADE TO PROTECT HUMAN HEALTH?

EPA requires that spills of 5,000 pounds or more of acetone be reported. To protect workers, the Occupational Safety and Health Administration (OSHA) has set a legal limit of 750 ppm of acetone in workroom air. The regulation means that the workroom air should contain no more than an average of 750 ppm of acetone over an 8-hour working shift or over a 40-hour workweek.

1.8 WHERE CAN I GET MORE INFORMATION?

If you have any more questions or concerns, please contact your community or state health or environmental quality department or:

Agency for Toxic Substances and Disease Registry
Division of Toxicology
1600 Clifton Road NE, E-29
Atlanta, Georgia 30333
404-639-6000

This agency can also tell you the location of occupational and environmental health clinics. These clinics specialize in the recognition evaluation, and treatment of illness resulting from exposure to hazardous substances.

2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective of the toxicology of acetone. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure - inhalation, oral, and dermal; and then by health effect - death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods - acute (14 days or less), intermediate (15-364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest- observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAEL have been classified into “less serious” or “serious” effects. “Serious” effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). “Less serious” effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, “less serious” LOAEL, or “serious” LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify

these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between “less serious” and “serious” effects. The distinction between “less serious” effects and “serious” effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user’s perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for acetone. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1989a), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

A User’s Guide has been provided at the end of this profile (see Appendix A). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

2.2.1 Inhalation Exposure

2.2.1.1 Death

In a retrospective mortality study of 948 employees (697 men, 251 women) of a cellulose fiber plant where acetone was used as the only solvent, no significant excess risk of death from any cause (all causes, malignant neoplasm, circulatory system disease, ischemic heart disease) compared with rates for the U.S. general population was found (Ott et al. 1983a, 1983b). The workers had been employed at the plant for at least 3 months to 23 years. Industrial hygiene surveys found that median time-weighted-average acetone concentrations were 380, 770, and 1,070 ppm based on job categories.

As shown in Table 2-l and Figure 2-1, high concentrations of acetone were required to produce death in animals. An 8-hour LC_{50} value of 21,091 ppm and a 4-hour LC_{50} value of 31,994 ppm were found for female rats (Pozzani et al. 1959). Inhalation exposure to acetone for a few hours has resulted in death in rats at concentrations ranging from 16, inhalation exposure to acetone for a few hours has resulted in death in rats at concentrations ranging from 16,000 to 50,600 ppm (Bruckner and Peterson 1981a; Smyth et al. 1962) and in guinea pigs from 10,000 to 50,000 ppm (Specht et al. 1939). In general, higher concentrations of acetone resulted in death sooner than lower concentrations. That very high concentrations of acetone are required to cause death of animals is reinforced by the fact that no deaths were reported for rats exposed to acetone at 4,210 for 8 hours to 126-129 ppm for 25 minutes (Haggard et al. 1944) or mice exposed to $\leq$84,194 ppm for 8 hours (Mashbitz et al. 1936). No studies were located regarding death of animals after intermediate- or chronic-duration inhalation exposure to acetone.

2.2.1.2 Systemic Effects

The systemic effects of inhalation exposure to acetone in humans and animals are discussed below. The highest NOAEL values and all LOAEL values for each systemic effect from each reliable study are recorded in Table 2-l and plotted in Figure 2-l.

Respiratory Effects. The only effect on the respiratory system observed in humans exposed to acetone vapors is irritation of the nose, throat, trachea, and lungs. The irritating properties of acetone in humans have been noted both in workers who were exposed to acetone occupationally (Raleigh and

TABLE 2-1. Levels of Significant Exposure to Acetone - Inhalation

Key to figure[a]	Species	Exposure duration/ frequency	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
ACUTE EXPOSURE							
Death							
1	Rat	1 d 4 hr/d				16000 (1/6 died)	Smyth et al. 1968
2	Rat	4 or 8 hr				21091 (LC_{50}-8 hr) 31994 (LC_{50}-4 hr)	Pozzani et al. 1959
3	Rat	1 d 3 hr/d				50600 (5/5 died)	Bruckner and Peterson 1981a
4	Gn pig	1 d 3-4 hr/d				50000 (8/8 died)	Specht et al. 1939
5	Gn pig	2 d 24 hr/d				10000 (5/5 died)	Specht et al. 1939
6	Gn pig	1 d 22-26 hr/d				20000 (8/9 died)	Specht et al. 1939
7	Gn pig	1 d 25 min-23.4 hr/d				21800 (2/10 died)	Specht et al. 1939
Systemic							
8	Human	6 d 6 hr/d	Resp		250 (irritation of nose and throat)		Matsushita et al. 1969a
			Hemato	250	500 (increased white blood cell count; decreased phagocytic activity of neutrophils)		
9	Human	1 d 3-5 min/d	Resp	200	500 (nose and throat irritation)		Nelson et al. 1943
10	Human	2-3 d 8 hr/d	Resp		901 (throat and nose irritation)		Raleigh and McGee 1972

TABLE 2-1. Levels of Significant Exposure to Acetone - Inhalation *(continued)*

Key to figure[a]	Species	Exposure duration/ frequency	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
11	Human	1 d 6 hr/d	Resp		100 (irritation of nose, throat and trachea)		Matsushita et al. 1969b
			Hemato	250	500 (increased white blood cell count; decreased phagocytic activity of neutrophils)		
12	Human	1 d 2 hr/d	Hemato Hepatic Renal	500 500 500			DiVincenzo et al. 1973
13	Human	1 d 2 min- 4 hr/d	Resp		12000 (throat and lung irritation)		Ross 1973
14	Human	7 d 8 hr/d	Resp		1006 (irritation of nose and throat)		Raleigh and McGee 1972
15	Rat	14 d 7 d/wk 6 hr/d Gd 6-19	Other	2200	11000 (significantly reduced body weight [7%], uterine weight [19%] and extra-gestational weight gain [36%] of dams)		NTP 1988
16	Rat	2 wk 5 d/wk 4 hr/d	Other (body weight)	16000			Goldberg et al. 1964
17	Gn pig	1 d 3-8.75 hr/d	Resp			50000 (pulomonary congestion and hemorrhage)	Specht et al. 1939
			Hepatic		50000 (mild fatty deposition)		
			Renal			50000 (congestion and distention of glomeruli)	
			Other			50000 (congestion and hemorrhage of spleen)	

TABLE 2-1. Levels of Significant Exposure to Acetone - Inhalation *(continued)*

Key to figure[a]	Species	Exposure duration/ frequency	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
18	Gn pig	1 d 22-26 hr/d	Resp			20000 (marked congestion and hemorrhage of lungs)	Specht et al. 1939
			Hepatic			20000 (fatty liver in guinea pigs that died)	
			Renal			20000 (distention of glomerular capsule)	
			Other			20000 (marked congestion and hemorrhage of spleen)	
19	Gn pig	2 d 24 hr/d	Resp			10000 (lung congestion in guinea pigs that died)	Specht et al. 1939
			Hepatic			10000 (fatty liver in guinea pigs that died)	
			Renal			10000 (renal tubular distention)	
			Other			10000 (congestion of spleen)	
20	Mouse	1 or 5 d 0.5 hr/d	Resp	6000			Schaper and Brost 1991
21	Mouse	1 d 10 min/d	Resp		77516 (RC_{50} for sensory irritation)		Kane et al. 1980
22	Mouse	12 d 7 d/wk 6 hr/d Gd 6-17	Hepatic	2200	6600 (significantly increased absolute and relative liver weight of dams)		NTP 1988
			Other (body weight)	6600			

TABLE 2-1. Levels of Significant Exposure to Acetone - Inhalation *(continued)*

Key to figure[a]	Species	Exposure duration/ frequency	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
Immunological							
23	Human	6 d 6 hr/d		250	500 (increased white blood cell count; decreased phagocytic activity of neutrophils)		Matsushita et al. 1969a
24	Human	1 d 6 hr/d		250	500 (increased white blood cell count; decreased phagocytic activity of neutrophils)		Matsushita et al. 1969b
Neurological							
25	Human	2-3 d 8 hr/d		901			Raleigh and McGee 1972
26	Human	7 d 8 hr/d			1006 (headache, light-headedness)		Raleigh and McGee 1972
27	Human	1 d 2 min-4 hr/d				12000 (unconsciousness, dizziness, unsteadiness, confusion, headache)	Ross 1973
28	Human	1-8 hr				21049 (signs of narcosis in 3-6 hours, loss of righting reflex in 8 hours)	Haggard et al. 1944
29	Human	1 d 6 hr/d			250 (lack of energy, general weakness)		Matsushita et al. 1969b
30	Human	1 d 4 hr/d			237[b] (increases in response and % false negatives in auditory discrimination; increased anger, hostility)		Dick et al. 1989

TABLE 2-1. Levels of Significant Exposure to Acetone - Inhalation *(continued)*

Key to figure[a]	Species	Exposure duration/ frequency	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
31	Human	6 d 6 hr/d			250 (delayed visual reaction time, headache, lack of energy, weakness)		Matsushita et al. 1969a
32	Human	4-8 hr			1000 (subjective symptoms of tension, tiredness, complaints and annoyance, not otherwise specified)		Seeber et al. 1992
33	Rat	1 d 3 hr/d				12600 (CNS depression measured by unconditioned performance and reflex tests)	Bruckner and Peterson 1981a
34	Rat	2 wk 5 d/wk 4 hr/d		3000		6000 (inhibition of avoidance behavior in 38% of the rats)	Goldberg et al. 1964
35	Rat	5 min- 8 hr		4210		10524 (signs of narcosis-loss of coordination in 100-250 minutes)	Haggard et al. 1944
36	Gn pig	1 d 25 min- 23.4 hr/d				21800 (narcosis, coma, paralysis)	Specht et al. 1939

TABLE 2-1. Levels of Significant Exposure to Acetone - Inhalation *(continued)*

Key to figure[a]	Species	Exposure duration/ frequency	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
37	Mouse	1 d		1000		3000 (10% decreased response to food presentation in a fixed interval operant behavioral test)	Glowa and Dews 1987
38	Mouse	4 hr		2032		2580 (39% decrease in duration of immobility in behavioral despair swimming test p<0.05)	DeCeaurriz et al. 1984
39	Mouse	1 d 6 hr/d				11000 (severe narcosis)	NTP 1988
40	Mouse	4 hr				16839 (drowsiness, staggering, prostration, clonic movements of hind legs, and deep narcosis)	Mashbitz et al. 1936
Developmental							
41	Rat	14 d 7 d/wk 6 hr/d Gd 6-19		2200	11000 (decreased fetal weight)		NTP 1988
42	Mouse	12 d 7 d/wk 6 hr/d Gd 6-17		2200		6600 (significantly increased incidence of late resorption, decreased fetal weight, reduced sternabral ossification)	NTP 1988
Reproductive							
43	Human	1 d 7.5 hr/d			1000 (shortened menstrual cycle)		Stewart et al. 1975

TABLE 2-1. Levels of Significant Exposure to Acetone - Inhalation *(continued)*

Key to figure[a]	Species	Exposure duration/ frequency	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
44	Rat	14 d 7 d/wk 6 hr/d Gd 6-19		11000			NTP 1988
45	Mouse	12 d 7 d/wk 6 hr/d Gd 6-17		6600			NTP 1988
INTERMEDIATE EXPOSURE							
Systemic							
46	Human	6 wk 2-5 d/wk 1-7.5 hr/d	Resp Cardio Hemato Hepatic Renal	1250 1250 1250 1250 1250			Stewart et al. 1975
47	Rat	2-8 wk 5 d/wk 3 hr/d	Resp Cardio Hepatic Renal	19000 19000 19000 19000			Bruckner and Peterson 1981b
Neurological							
48	Human	6 wk 2-5 d/wk 1-7.5 hr/d			1250[c] (increased visual evoked response)		Stewart et al. 1975
49	Rat	2-8 wk 5 d/wk 3 hr/d		19000			Bruckner and Peterson 1981b

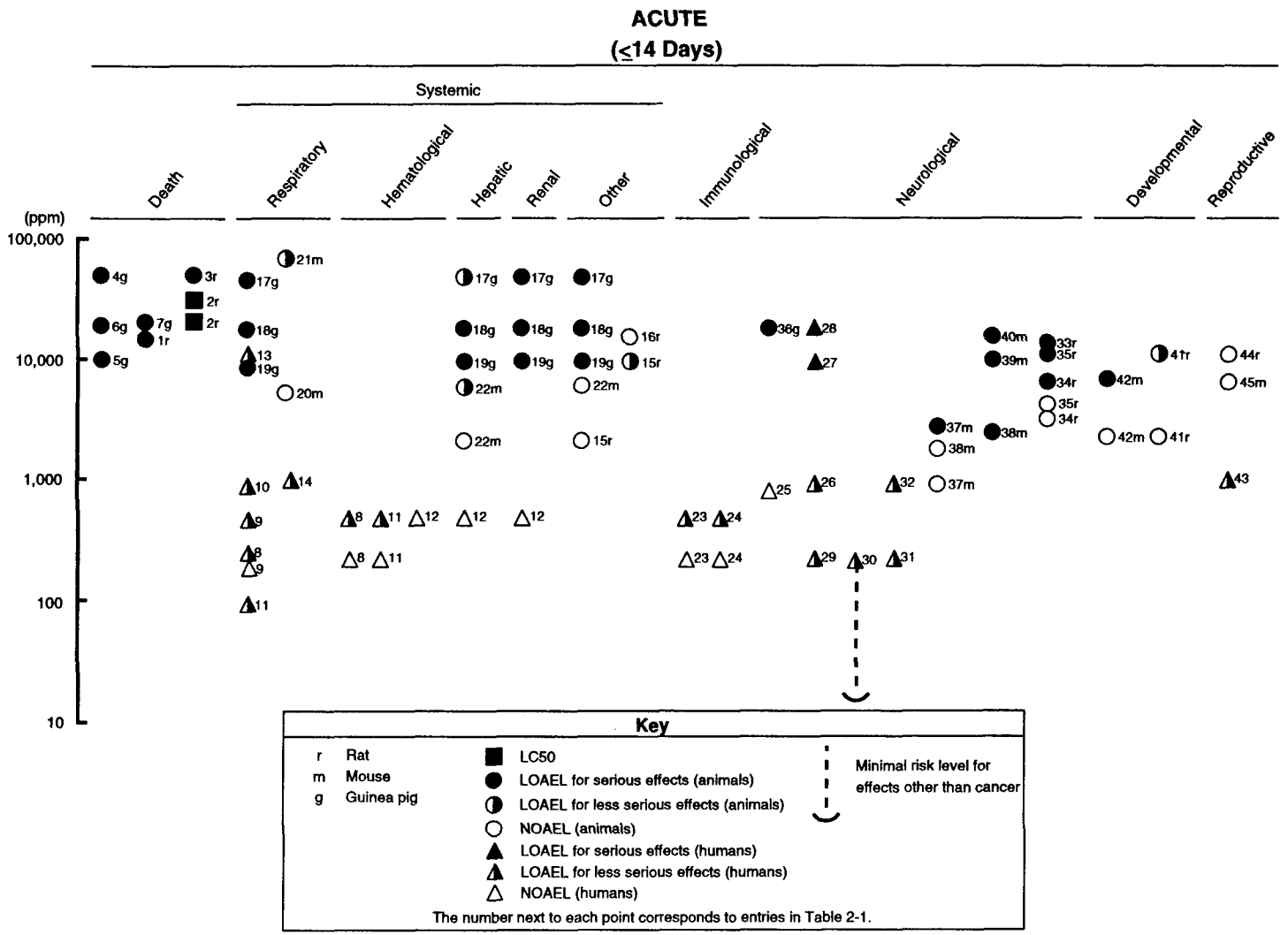
TABLE 2-1. Levels of Significant Exposure to Acetone - Inhalation *(continued)*

Key to figure[a]	Species	Exposure duration/ frequency	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
CHRONIC EXPOSURE							
Systemic							
50	Human	3 mo- 23 yr 5 d/wk 8 hr/d (occup)	Hemato Hepatic	1070 1070			Ott et al. 1983a; 1983c

[a]The number corresponds to entries in Figure 2-1.
[b]Used to derive an acute inhalation minimal risk level (MRL) of 26 ppm; concentration divided by an uncertainty factor of 9 (3 for use of a minimal LOAEL and 3 for human variability)
[c]Used to derive intermediate and chronic inhalation MRLs of 13 ppm; concentration divided by an uncertainty factor of 100 (10 for use of a LOAEL and 10 for human variability)

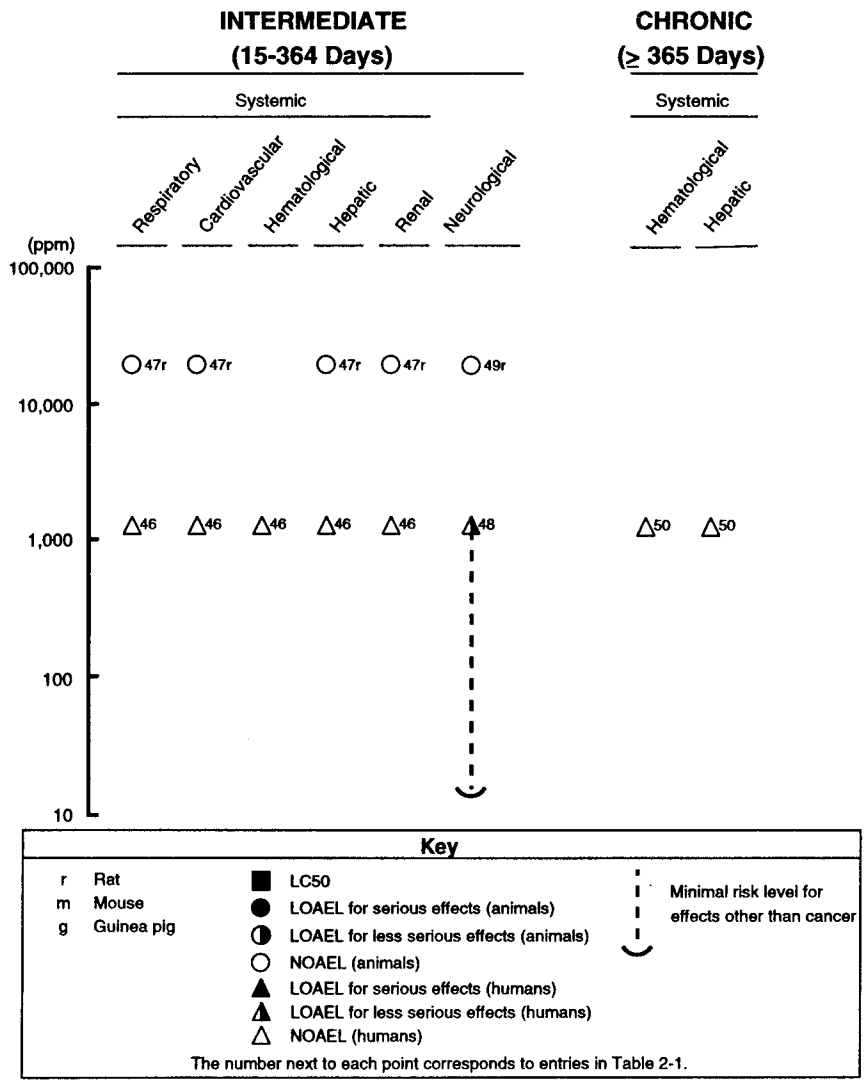
Cardio = cardiovascular; CNS = central nervous system; d = day(s); Gd = gestation day(s); Gn pig = guinea pig; Hemato = hematological; hr = hour(s); LC_{50} = lethal concentration, 50% kill; LOAEL = lowest-observed-adverse-effect level; min = minute(s); NOAEL = no-observed-adverse-effect level; occup = occupational; RC_{50} = the concentration necessary to detect a 50% decrease in respiratory rate; Resp = respiratory; wk = week(s); yr = year(s)

FIGURE 2-1. Levels of Significant Exposure to Acetone - Inhalation



142078-1

FIGURE 2-1. Levels of Significant Exposure to Acetone - Inhalation *(continued)*



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McGee 1972; Ross 1973) and in volunteers under controlled laboratory conditions (Matsushita et al. 1969a, 1969b; Nelson et al. 1943). Complaints of irritation were reported by workers with average exposures to acetone in the workroom of ≥901 ppm (Raleigh and McGee 1972; Ross 1973). In controlled situations, the volunteers had been asked to give their subjective complaints, and some of the volunteers reported that exposure to 100 ppm for 6 hours was irritating, with more subjects reporting irritation at increasing exposure levels (Matsushita et al. 1969b). Subjective symptoms also included the loss of the ability to smell acetone as exposure proceeded. In another controlled experiment, the majority of subjects, although exposed for only 3-5 minutes, estimated that they could tolerate an exposure level of 200 ppm for an 8-hour workshift (Nelson et al. 1943). Pulmonary function testing of volunteers exposed <1,250 ppm acetone intermittently for various durations in a complex protocol revealed no abnormalities caused by the exposure (Stewart et al. 1975). The volunteers did experience throat irritation sporadically.

Exposure of animals to much higher concentrations of acetone than those reported in humans has resulted in respiratory effects. Pulmonary congestion, edema, and hemorrhage of the lungs were observed in guinea pigs that died after exposure to 10,000 ppm continuously for 1 or 2 days, to 20,000 ppm continuously for 1 day, or to 50,000 ppm for a few hours (Specht et al. 1939). The congestion and edema were attributed to the irritating effects of acetone on the mucosa. The hemorrhage may have been a consequence of death. Respiratory rates also decreased in the guinea pigs during exposures, but the decrease was probably a consequence of the narcotic effects of acetone (see Section 2.2.1.4). In mice exposed to acetone for 10 minutes, the calculated concentration of acetone that decreased the respiratory rate 50% (RC_{50}) was 77,516 ppm (Kane et al. 1980). The decrease in respiratory rate was considered to be due to sensory irritation, but adaptation to the irritant properties developed. The RC_{50} for acetone was higher than the values calculated for other solvents, indicating that acetone is a weak irritant. In mice exposed to 6,000 ppm acetone for 0.5 hours/day for 1 or 5 days, no effects on the time of inspiration, time of expiration, time between breaths, or tidal volume were found (Schaper and Brost 1991). In addition, acetone exposure caused no changes in lung weight, lung volume displacement, or histological evidence of pulmonary pathology.

Histological examination of the lungs of rats exposed intermittently to a high concentration of acetone (19,000 ppm) for 2-8 weeks revealed no evidence of treatment-related lesions (Bruckner and Peterson 1981b).

Cardiovascular Effects. Information regarding cardiovascular effects in humans following inhalation exposure to acetone is limited. High pulse rates (120-160/minute) were commonly found in patients exposed to acetone by inhalation and/or dermally after application of casts for which acetone was used in the setting solution (Chatterton and Elliott 1946; Hift and Pate1 1961; Pomerantz 1950; Renshaw and Mitchell 1956). In a controlled laboratory study using a complex protocol, electrocardiography of volunteers exposed to ≤1,250 ppm acetone intermittently for various durations revealed no alterations, compared with their preexposure electrocardiograms (Stewart et al. 1975). A retrospective mortality study of 948 workers (697 men, 251 women) employed for at least 3 months to 23 years at a cellulose fiber plant where acetone was used as the only solvent found no significant excess risk of death from circulatory system disease or ischemic heart disease compared with rates for the U.S. general population (Ott et al. 1983a, 1983b). Industrial hygiene surveys found that median time-weighted-average acetone concentrations were 380,770, and 1,070 ppm based on job categories.

Reduced heart rates were observed in guinea pigs exposed to various high concentrations of acetone for various acute durations (Specht et al. 1939), but were probably a consequence of the narcotic effects of acetone (Section 2.2.1.4). Necropsy of the guinea pigs revealed no effects on the heart, but histological examination was not performed. Histological examination of the hearts of rats exposed intermittently to a high concentration of acetone (19,000 ppm) for 2-8 weeks revealed no evidence of treatment-related lesions (Bruckner and Peterson 1981b).

Gastrointestinal Effects. Case reports have described vomiting of blood and gastrointestinal hemorrhage in patients who had hip casts applied with acetone present in the setting fluid (Chatterton and Elliott 1946; Fitzpatrick and Claire 1947; Harris and Jackson 1952; Hift and Pate1 1961; Pomerantz 1950; Renshaw and Mitchell 1956; Strong 1944). As the vomitus contained blood several hours after vomiting first commenced, the gastrointestinal hemorrhage may have been due to the trauma of repeated vomiting. These patients had a strong odor of acetone in their breath, and acetone was detected in the urine and blood. These patients were exposed to acetone by inhalation during cast application and from evaporation from the casts after the applications. In addition, the possibility of contribution from dermal exposure could not be ruled out. In one case, exposure was considered to be mainly dermal (Hift and Pate1 1961).

Necropsy of guinea pigs that died after exposure to various high concentrations of acetone for various acute durations revealed no effects on the stomach (Specht et al. 1939), but histological examination was not performed.

Hematological Effects. In a health evaluation survey of 168 men and 77 women employed at a cellulose fiber production plant where acetone was used as the only solvent, all hematological parameters were within normal limits (Ott et al. 1983a, 1983c). The workers had been employed at the plant for at least 3 months to 23 years. Industrial hygiene surveys found median time-weighted-average acetone concentrations of 380, 770, and 1,070 ppm, based on job categories. Hematological effects have been observed in humans after inhalation exposure to acetone in controlled laboratory studies of volunteers. Statistically significant increased white blood cell counts and decreased phagocytic activity of neutrophils, compared with controls, were observed in the volunteers after a 6-hour exposure or repeated 6-hour exposures for 6 days to 500 ppm (Matsushita et al. 1969a, 1969b). No significant difference was seen in hematological parameters in the volunteers exposed to 250 ppm compared with controls. In contrast, hematological findings were within normal limits in volunteers exposed to 500 ppm for 2 hours (DiVincenzo et al. 1973) or ≤1,250 ppm acetone repeatedly for l-7.5 hours/day for as long as 6 weeks (Stewart et al. 1975).

In animals, no studies were located regarding effects on the formed elements of the blood after inhalation exposure to acetone.

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans or animals after inhalation exposure to acetone.

Hepatic Effects. No indication that acetone caused hepatic effects in humans was found in controlled studies of volunteers. Clinical chemistry parameters indicative of liver injury (e.g., serum alanine aminotransferase, aspartate aminotransferase, lactic dehydrogenase, alkaline phosphatase, ornithine carbamoyl transferase, cholesterol, triglycerides, bilirubin, lipids, etc.) were within normal limits in volunteers exposed to acetone at concentrations of 500 ppm for 2 hours (DiVincenzo et al. 1973) or 11,250 ppm intermittently for various durations (Stewart et al. 1975). In a health evaluation survey of 168 men and 77 women employed for at least 3 months to 23 years at a cellulose fiber production plant where acetone was used as the only solvent, all clinical blood chemistry parameters (aspartate aminotransferase, alanine aminotransferase, lactic dehydrogenase, alkaline phosphatase, total

bilirubin, and albumin) were within normal limits (Ott et al. 1983a, 1983c). Industrial hygiene surveys found median time-weighted-average acetone concentrations of 380, 770, and 1,070 ppm, based on job categories.

Fatty deposits were found in the livers upon autopsy of guinea pigs that died after exposure to high concentrations of acetone for various acute durations (Specht et al. 1939). In contrast, intermittent exposure of rats to a high concentration of acetone (19,000 ppm) for 2-8 weeks did not produce signs of liver toxicity, assessed by the measurement of serum aspartate aminotransferase, lactic dehydrogenase, liver weights, and histological examination of the liver (Bruckner and Peterson 1981b).

Inhalation exposure to acetone at lower concentrations does not appear to be toxic to the liver of animals; however, acetone potentiates the hepatotoxicity induced by some other chemicals (see Section 2.6). The mechanism by which acetone exerts the potentiation is through the induction or increased activity of liver microsomal monooxygenases, particularly enzymes associated with cytochrome P-450IIEl (see Sections 2.35 and 2.6). Most of the studies showing enzyme induction have been conducted by the oral route (see Section 2.2.2.2). In acute inhalation studies in rats, acetone exposure resulted in statistically significant increases in the liver concentration of cytochrome P-450, the activity of ethoxycoumarin O-deethylase (associated with P-450IIBl), and the activity of glutathione-S-transferase, and decreased the liver free glutathione content (Brondeau et al. 1989; Vainio and Zitting 1978). Induction of microsomal enzymes is considered a normal physiological response to xenobiotics, rather than an adverse effect.

In a developmental study, mice exposed intermittently to 6,600 ppm acetone on gestational days 6-19 had significantly increased absolute and relative liver weights compared with controls ($p<0.05$) (NTP 1988). Increased liver weight is considered a sign of maternal toxicity in developmental studies. The increased liver weight could have been associated with enzyme induction.

Renal Effects. No indication that acetone caused renal effects in humans was found in controlled studies of volunteers. Clinical blood chemistry parameters indicative of kidney injury (e.g., blood urea nitrogen, uric acid) and urinalysis parameters were within normal limits in volunteers exposed to acetone at concentrations of 500 ppm for 2 hours (DiVincenzo et al. 1973) or ≤1,250 ppm intermittently for various durations (Stewart et al. 1975).

The only indication that inhalation exposure to acetone causes renal effects in animals was the consistent finding of congestion or distention of renal tubules or glomeruli in guinea pigs that died after exposure to high concentrations of acetone for various acute durations (Specht et al. 1939). Rats exposed intermittently to 19,000 ppm for ≤8 weeks had significantly decreased kidney weights ($p<0.0l$) after 4 weeks of exposure compared with controls, but not after 2 or 8 weeks of exposure or at 2 weeks postexposure (Bruckner and Peterson 1981b). Blood urea nitrogen levels were not affected by acetone exposure, and no evidence of histological changes in the kidneys were found. In the absence of other evidence of renal toxicity, the sporadically reduced kidney weight cannot be considered an adverse effect.

Derma/Ocular Effects. Eye irritation is a common complaint of workers exposed to acetone vapors occupationally (Raleigh and McGee 1972) and in volunteers exposed under controlled conditions (Matsushita et al. 1969a, 1969b; Nelson et al. 1943; Ross 1973). In a report of the experience at the Tennessee Eastman Corporation on acetone concentrations not associated with injury presented at the American Conference of Governmental Industrial Hygienists (ACGIH) Tenth Annual Meeting, it was noted that acetone is mildly irritating to the eyes at 2,000-3,000 ppm, with no irritation persisting after exposure ceases (Sallee and Sappington 1949). Lacrimation has also been observed in guinea pigs exposed to acetone vapors (Specht et al. 1939). Since eye irritation is due to direct contact of the eyes with the vapor rather than a true systemic effect of inhalation of the vapor, this and other dermal/ocular effects resulting from direct contact with acetone are discussed in Section 2.3.3.

Other Systemic Effects. No studies were located regarding other systemic effects in humans after inhalation exposure to acetone.

Other systemic effects observed in animals after inhalation exposure to acetone include body weight changes. In a developmental study, rats exposed to acetone at 11,000 ppm, but not mice exposed to 6,600 ppm, intermittently during gestation had significantly ($p<0.05$) reduced body weight gain from gestational day 14 onward and reduced extragestational body weight on gestational day 20 (NTP 1988). However, in a behavioral study, no effect on body weight gain was observed in female rats exposed to 16,000 ppm intermittently for 2 weeks (Goldberg et al. 1964). It is possible that the condition of pregnancy made the rats more susceptible to body weight reduction. In addition, marked congestion and hemorrhage of the spleen were observed upon autopsy of guinea pigs that died after

exposure to various high concentrations of acetone for various acute durations (Specht et al. 1939). These effects could have been the consequence of death.

2.2.1.3 Immunological Effects

The only information regarding immunological effects in humans after inhalation exposure to acetone is the finding of statistically significant increased white blood cell counts, increased eosinophil counts, and decreased phagocytic activity of neutrophils in volunteers exposed to 500 ppm for a single 6-hour exposure or intermittently for 6 days (Matsushita et al. 1969a, 1969b). No significant difference in these parameters was seen in the volunteers exposed to 250 ppm compared with controls. Hematological parameters, including total white cell counts and differential white cell counts, were within normal limits in other volunteers exposed to 500 ppm for 2 hours (DiVincenzo et al. 1973) or ≤1,250 ppm acetone intermittently for durations in a study with a complex protocol (Stewart et al. 1975); however, these investigators did not examine the phagocytic activity of neutrophils. The NOAEL value of 250 ppm and LOAEL value of 500 ppm are recorded in Table 2-l and plotted in Figure 2- 1.

No studies were located regarding immunological effects in animals after inhalation exposure to acetone.

2.2.1.4 Neurological Effects

Case reports have described patients who became comatose or collapsed after hip casts were applied with acetone present in the setting fluid (Chatterton and Elliott 1946; Fitzpatrick and Claire 1947; Harris and Jackson 1952; Renshaw and Mitchell 1956; Strong 1944). In addition, a woman experienced headache, dizziness, weakness, difficulty speaking, and depression after a cast containing acetone had been applied (Pomerantz 1950). These patients had a strong odor of acetone in their breath, and acetone was detected in the urine and blood. These patients were exposed to acetone by inhalation during cast application and from evaporation from the casts after the applications. In addition, the possibility of contribution from dermal exposure could not be ruled out. In another case of neurological effects (drowsiness, fretfulness, irritability, restlessness, uncoordinated hand movement, nystagmus) developing after application of a cast, exposure was considered to be mainly dermal because an airblower was used continuously during the application to dissipate the fumes (Hift and

Pate1 1961). However, because the patient had kept his head under a blanket, some inhalation of acetone evaporating from the cast may have occurred.

Workers exposed to acetone in the past commonly experienced neurological effects. In an on-site medical appraisal of nine workers, in which the time-weighted average exposure concentration was 1,006 ppm, three of the workers mentioned headache and lightheadedness as subjective symptoms (Raleigh and McGee 1972). In another on-site medical appraisal of four workers, in which the time-weighted average exposure concentration was 901 ppm, none of the workers complained of neurological effects (Raleigh and McGee 1972). The medical examinations included the Romberg test, finger-to-nose test, and observations for nystagmus (involuntary rapid movement of the eyeball). These tests revealed no neurobehavioral effects in either study. Such symptoms as unconsciousness, dizziness, unsteadiness, confusion, and headache were experienced by seven workers exposed to >12,000 ppm acetone while cleaning out a pit containing acetone, that had escaped from nearby tanks (Ross 1973). The degree of the symptoms varied depending on the length of time that the workers had spent in the pit (2 minutes to 4 hours).

Neurological and behavioral effects have also been documented in volunteers tested under controlled laboratory conditions. These effects included general lack of energy and weakness, headache, delayed visual reaction time (Matsushita et al. 1969a, 1969b); subjective symptoms of tension, tiredness, complaints (not otherwise specified), and annoyance (Seeber and Kiesswetter 1991; Seeber et al. 1992); increases in response and the percent false negatives in auditory discrimination tests and increases in anger and hostility (Dick et al. 1989); and increased visual evoked response (Stewart et al. 1975). Other neurological and neurobehavioral tests (e.g., electroencephalography, choice reaction time, visual vigilance, dual task, memory scanning, postural sway, Romberg test, or heel-to-toe test) were also conducted on these volunteers, but acetone exposure had no effect on these parameters. The relationship between concentration and duration of exposure on the development of narcosis was demonstrated in volunteers exposed to acetone at 21,049-84,194 ppm for 1-8 hours (Haggard et al. 1944). As the concentration increased, the time to observations of signs of narcosis (not otherwise described), loss of righting reflex, and loss of cornea1 reflex decreased. It should be noted that these concentrations of acetone are extremely high, and exposure to lower concentrations of acetone for shorter durations has resulted in unconsciousness in some workers, as discussed above. Based on a LOAEL of 237 ppm for 4 hours for neurobehavioral effects in humans (Dick et al. 1989), an acute inhalation MRL of 26 ppm was calculated as described in footnote “b” in Table 2-l. Based on a

LOAEL of 1,250 ppm for neurological effects in a 6-week study (Stewart et al. 1975), intermediate and chronic inhalation MRLs of 13 ppm were calculated as described in footnote “c” in Table 2-l.

Narcotic effects have been observed in animals exposed acutely to acetone vapors. The narcotic effects observed in animals after inhalation exposure to acetone depend upon the duration and the magnitude of exposure. The narcotic effects appear to proceed through several stages: drowsiness, incoordination, loss of autonomic reflexes, unconsciousness, respiratory failure, and death as concentrations and durations increase. The acute data suggest that concentrations >8,000 ppm generally are required to elicit overt signs of narcosis, although neurobehavioral effects, when assessed by specific behavioral tests, have been observed at lower concentrations. The relationship between concentration and duration of exposure on the development of narcosis was demonstrated in rats exposed to acetone at 2,105-126,291 ppm for 5 minutes to 8 hours (Haggard et al. 1944). While exposure to 2,105 or 4,210 ppm for 8 hours resulted in no signs of narcosis or effects on righting reflex or cornea1 reflex, these effects were observed at higher concentrations. At increasing concentrations ≥10,524 ppm, the time to observations of signs of narcosis, loss of righting reflex, and loss of cornea1 reflex decreased. The responses were correlated with blood acetone levels. Similar concentration- and duration-response relationships were found in mice exposed to 16,839-84,194 ppm acetone for up to 4 hours (Mashbitz et al. 1936). Neurological responses included drowsiness, staggering, prostration, clonic movements of hind legs, and deep narcosis. Narcosis, evidenced by decreased respiratory and heart rates, paralysis, and coma were observed in guinea pigs exposed to 21,800 ppm continuously for periods ranging from 25 minutes to 24 hours (Specht et al. 1939). The degree of narcosis increased as the exposure duration increased. In a developmental study, virgin and pregnant mice experienced severe narcosis after a single 6-hour exposure to 11,000 ppm on the first day, but narcosis was no longer observed when the exposure was lowered to 6,600 ppm 6 hours/day for the rest of the study (NTP 1988).

Neurobehavioral effects, indicative of narcosis, have been observed in rats, mice, and baboons acutely exposed to acetone vapors. These effects include central nervous system depression measured by five tests of unconditioned performance and reflex in rats (Bruckner and Peterson 1981a), decreased operant behavior evaluated by a multiple fixed ratio-fixed interval schedule of food reinforcement in rats (Geller et al. 1979b), inhibition of avoidance behavior and escape response in rats (Goldberg et al. 1964), decreased response to food presentation in mice (Glowa and Dews 1987), decreased duration of immobility in a behavioral despair swimming test in mice (DeCeaurriz et al. 1984), and incoordination

in a “match to sample” operant behavioral test in baboons (Geller et al. 1979a). In these studies, the animals recovered from the neurobehavioral effects as exposure continued, indicating adaptation or tolerance, or after exposure ceased, demonstrating the reversibility of these effects. The length of the recovery period was generally related to the level of exposure (Bruckner and Peterson 1981a; Glowa and Dews 1987). In the experiments of Geller et al. (1979a, 1979b), only four baboons and three rats were studied, precluding meaningful statistical analysis, and only two of the four baboons exhibited the effects. The rats were exposed to 150 ppm for 0.5-4 hours, and the baboons were exposed to 500 ppm continuously for 7 days. Intermediate-duration intermittent exposure of rats to 19,000 ppm acetone resulted in a statistically significant decrease ($p<0.02$) in absolute brain weight, but no exposure-related histological lesions (Bruckner and Peterson 1981b). Thus, based on the available data, the neurological effects of acetone are reversible and cannot be attributed to histologically observable changes in the brains of animals or to electroencephalographic changes in humans.

The highest NOAEL values and the LOAEL values for neurological effects from each reliable study are recorded in Table 2-l and plotted in Figure 2- 1.

2.2.1.5 Reproductive Effects

Information regarding reproductive effects in humans after inhalation exposure to.acetone is limited. Premature menstrual periods were reported by three of four women exposed to 1000 ppm acetone for 7.5 hours in a laboratory study of volunteers (Stewart et al. 1975). The shortening of the menstrual cycle was considered to be possibly due to the acetone exposure. Women workers in a Russian factory where workroom levels of acetone ranged from 14 to 126 ppm were reported to have statistically significantly increased incidences of pregnancy complications, including miscarriage, toxicosis (not otherwise described), decreased hemoglobin levels and hypotension, and “weakness of labor activity,” compared with controls (Nizyaeva 1982). However, the number of women studied, further description of the exposed and control groups (such as age, smoking history, use of alcohol), and description of workroom monitoring methods and statistical methods were not reported. Therefore, no conclusions can be made from this report. In a epidemiological study of the pregnancy outcome among 556 female laboratory workers, no statistically significant difference in the incidence of miscarriage was found between those exposed to a variety of solvents including acetone and those not exposed to solvents (Axelsson et al. 1984).

No reproductive effects (i.e., no effects on number of implants/litter, percent live pups/litter, or mean percent resorptions/litter) were observed in rats or mice in an inhalation developmental study (NTP 1988). No studies were located regarding reproductive effects in male animals, histological effects on reproductive organs of male or female animals, or the reproductive outcomes and other indices of reproductive toxicity in animals after inhalation exposure to acetone. The NOAEL values for reproductive effects in female rats and mice and the LOAEL value for premature menstrual periods in humans are recorded in Table 2-1 and plotted in Figure 2-1.

2.2.1.6 Developmental Effects

Information regarding developmental effects in humans after inhalation exposure to acetone is limited. Statistically significant increased incidences of developmental effects, such as, intrauterine asphyxia of fetuses and decreased weight and length of neonates, were reported for women workers in a Russian factory, where workroom levels of acetone ranged from 14 to 126 ppm (Nizyaeva 1982). However, the number of women studied, further description of the exposed and control groups (such as age, smoking history, use of alcohol), and description of workroom monitoring methods and statistical methods were not reported. Therefore, no conclusions can be made from this report. In a epidemiological study of the pregnancy outcome among 556 female laboratory workers, no statistically significant differences in the incidences of miscarriage, perinatal death rate, or malformations were found between those exposed to a variety of solvents, including acetone, and those not exposed to solvents (Axelsson et al. 1984).

In a development study in rats exposed intermittently to acetone during gestation, the only effect was a slight, but significant ($p<0.05$), decreased mean male and female fetal body weight at 11,000 ppm (NTP 1988). It should be noted that the dams exposed at this level had significantly ($p<0.05$) reduced body weight during gestation, reduced uterine weight, and reduced extragestational weight on gestational day 20. No effects were seen on sex ratio, incidence of fetal variations, reduced ossification sites, or mean fetal variations. The percent of litters with at least one fetal malformation was higher in the 11,000 ppm group than in the control group, but no statistically significant increased incidences of fetal malformations were observed. In mice similarly exposed during gestation, however, there was a slight, but significant ($p<0.05$) increase in percent late resorptions, decrease in mean male and female fetal weights, and increase in the incidence of reduced sternebral ossification in the 6,600 ppm group. The only evidence of maternal toxicity at this exposure level was statistically

significant increased absolute and relative liver weight. No effects were found on the number of implantations per litter, percent live fetuses/litter, sex ratio, incidence of malformations or skeletal variations combined. The NOAEL values and LOAEL values for developmental effects in rats and mice are recorded in Table 2- 1 and plotted in Figure 2-l.

2.2.1.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or animals after inhalation exposure to acetone. Genotoxicity studies are discussed in Section 2.4.

2.2.1.8 Cancer

In a retrospective mortality study of 948 employees (697 men, 251 women) of a cellulose fiber plant where acetone was used as the only solvent, no significant excess risk of death from any cause, including malignant neoplasm, was found when compared with rates for the U.S. general population (Ott et al. 1983a, 1983b). The workers had been employed at the plant for at least 3 months to 23 years. Industrial hygiene surveys found that median time-weighted-average acetone concentrations were 380, 770, and 1,070 ppm, based on job categories.

No studies were located regarding cancer in animals after inhalation exposure to acetone.

2.2.2 Oral Exposure

2.2.2.1 Death

The 1991 Annual Report of the American Association of Poison Control Centers National Data Collection System documented 1,137 incidents of human exposure to acetone (Litovitz et al. 1992). Of these incidents, 1,124 were due to accidental or intentional ingestion (the others were not clearly specified). No fatalities were reported, only three cases had a major medical problem, 364 were treated in a health care facility, 233 cases were referred to hospitals but had no effects, 367 cases

suffered minor effects, and 39 suffered from moderate effects. None of the major, minor, or moderate effects were further described, and the outcomes of the remainder of the incidents were not reported.

As seen in Table 2-2 and Figure 2-2, acute lethal dose (LD_{50}) values were located for rats, mice, and guinea pigs. In general, the lethality of acetone decreases with the age of the rats (Kimura et al. 1971). A higher LD_{50} value was found for young adult rats than for older adult rats, but the difference was not statistically significant. Higher LD_{50} values were found for Wistar rats (Smyth et al. 1962) and Nelson rats (Pozzani et al. 1959) than for Sprague-Dawley rats (Kimura et al. 1971). The LD_{50} value determined by Freeman and Hayes (1985), who also used Sprague-Dawley rats, is in line with values for 14-day-old and young adult Sprague-Dawley rats. An oral LD_{50} value of 5,250 mg/kg was found for male ddY mice (Tanii et al. 1986), and an oral LD_{50} value of 3,687 mg/kg was found for male guinea pigs (Striegel and Carpenter 1961). In a study to determine which doses to use in a developmental study, oral dosing of pregnant mice with acetone during gestation resulted in the death of one of four mice at 2,400 mg/kg/day, and the number of dead mice increased as the dose increased (EHRT 1987). No controls were used in the range-finding study. One of two rabbits given 7,844 mg/kg acetone by gavage died within 19 hours of dosing, two rabbits given 5,491 mglkg survived, while one rabbit given 3,922 mg/kg died in 96 hours (Walton et al. 1928). Oral doses of 7,500 or 8,000 mg/kg acetone were fatal to two puppies (Albertoni 1884). No controls were included in these studies, and the small numbers of animals used limits the reliability of the findings. Signs of narcosis usually precede death in animals (see Section 2.2.2.4). No information was located regarding the doses of acetone that could result in increased mortality after intermediate- or chronic-duration exposure.

2.2.2.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, hematological, musculoskeletal, hepatic, renal, or dermal/ocular effects in humans after oral exposure to acetone. The systemic effects in humans and animals after oral exposure to acetone are discussed below. The highest NOAEL values and the LOAEL values for each systemic effect from all reliable studies are recorded in Table 2-2 and plotted in Figure 2-2.

Respiratory Effects. Oral exposure of animals to acetone has not been shown to produce adverse respiratory effects. However, microsomes from the lungs of hamsters exposed to acetone in drinking

TABLE 2-2. Levels of Significant Exposure to Acetone - Oral

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
ACUTE EXPOSURE								
Death								
1	Rat	(G)	1 d 1x/d				1726 (LD_{50}-newborn rats) 4393 (LD_{50}-14-day old rats) 7138 (LD_{50}-young adult rats) 6667 (LD_{50}-older adult rats)	Kimura et al. 1971
2	Rat	(G)	once				9883 (LD_{50})	Pozzani et al. 1959
3	Rat	(G)	1 d 1x/d				8393 (LD_{50})	Smyth et al. 1962
4	Rat	(G)	1 d 1x/d				5800 (LD_{50})	Freeman and Hayes 1985
5	Gn pig	(G)	once				3687 (LD_{50})	Striegel and Carpenter 1961
6	Mouse	(G)	once				5250 (LD_{50})	Tanii et al. 1986
Systemic								
7	Human	(G)	once	Gastro		2241 (sore throat and erosion in mouth)		Gitelson et al. 1966
				Other			2241 (diabetes-like condition, hyperglycemia, polyuria, excessive thirst)	

TABLE 2-2. Levels of Significant Exposure to Acetone - Oral *(continued)*

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
8	Rat	(W)	14 d	Hemato	4312		6942 (bone marrow hypoplasia)	NTP 1991; Dietz et al. 1991
				Hepatic	8560			
				Renal	8560			
				Other (body weight)	8560			
9	Rat	(G)	1 d 1x/d	Other		5800 (temporary 15% loss of body weight)		Freeman and Hayes 1985
10	Rat	(GW)	1 d 1x/d	Hepatic	1961			Plaa et al. 1982
11	Rat	(GW)	3 d 2x/d	Hepatic	392			Plaa et al. 1982
12	Rat	(GO)	1 d 1x/d	Hepatic	871			Brown and Hewitt 1984
				Renal		871 (degeneration of apical microvilli in renal tubules)		
13	Rat	(W)	3-7 d	Other		3214 (reduced insulin stimulated glucose oxidation in epididymal fat)		Skutches et al. 1990
14	Rat	(GW)	2 d 3x in 2 d	Renal	1766			Valentovic et al. 1992
				Other (body weight)	1766			
15	Rat	(GO)	1 d 1x/d	Hepatic	1177			Charbonneau et al. 1986b

TABLE 2-2. Levels of Significant Exposure to Acetone - Oral *(continued)*

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
16	Mouse	(W)	14 d	Hepatic	1579	3896 (minimal to mild hepatocellular hypertrophy)		NTP 1991; Dietz et al. 1991
				Renal	12725			
				Other (body weight)	12725			
17	Mouse	(GW)	10 d Gd 6-15 1x/d	Other		3500 (reduced maternal body weight; [5%])		EHRT 1987
18	Mouse	(W)	10 d ad lib	Hepatic	1900			Jeffery et al. 1991
Neurological								
19	Human	(G)	once				2241 (coma, abnormal gait)	Gitelson et al. 1966
20	Rat	(G)	1 d 1x/d				5800 (prostration)	Freeman and Hayes 1985
Developmental								
21	Mouse	(GW)	10 d Gd 6-15 1x/d				3500 (decreased survival of pups)	EHRT 1987
Reproductive								
22	Mouse	(GW)	10 d Gd 6-15 1x/d				3500 (reduced reproduction index, increased gestation duration)	EHRT 1987

TABLE 2-2. Levels of Significant Exposure to Acetone - Oral *(continued)*

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
INTERMEDIATE EXPOSURE								
Systemic								
23	Rat	(GW)	93-95 d 1x/d	Resp	2500			American Biogenics Corp. 1986
				Cardio	2500			
				Gastro	2500			
				Hemato	500	2500 (increased hemoglobin, hematocrit, mean cell hemoglobin, mean cell volume, decreased platelets)		
				Musc/skel	2500			
				Hepatic	500	2500 (increased serum alamine aminotransferase)		
				Renal	100	500 (increased severity of age-related nephropathy in males)		
				Derm/oc	2500			
				Other (body weight)	2500			
24	Rat	(GW)	46-47 d 1x/d	Hemato	500	2500 (increased hemoglobin, hematocrit, mean cell volume)		American Biogenics Corp. 1986
				Hepatic	500	2500 (increased serum alanine aminotransferase)		
				Other (body weight)	2500			
25	Rat	(W)	12 wk	Other (body weight)	732			Spencer et al. 1978

TABLE 2-2. Levels of Significant Exposure to Acetone - Oral *(continued)*

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
26	Rat	(W)	6 wk	Other (body weight)	650			Ladefoged et al. 1989
27	Rat	(W)	13 wk	Resp	3400			NTP 1991; Dietz et al. 1991
				Cardio	3400			
				Gastro	3400			
				Hemato	200[b]	400 (mild macrocytic anemia)		
				Musc/skel	3400			
				Hepatic	3400			
				Renal	900	1700 (increased incidence and severity of nephropathy in males)		
				Derm/oc	3400			
				Other (body weight)	3400			
28	Mouse	(W)	13 wk	Resp	11298			NTP 1991; Dietz et al. 1991
				Cardio	11298			
				Gastro	11298			
				Hemato	11298			
				Musc/skel	11298			
				Hepatic	11298			
				Renal	11298			
				Derm/oc	11298			
				Other (body weight)	11298			
Neurological								
29	Rat	(GW)	93-95 d 1x/d		500	2500 (decreased brain weight, salivation)		American Biogenics Corp. 1986
30	Rat	(GW)	46-47 d 1x/d		500	2500 (excessive salivation)		American Biogenics Corp. 1986

TABLE 2-2. Levels of Significant Exposure to Acetone - Oral *(continued)*

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
31	Rat	(W)	6 wk			650 (decreased motor nerve conduction velocity)		Ladefoged et al. 1989
32	Rat	(W)	12 wk		732			Spencer et al. 1978
33	Rat	(W)	13 wk		3400			NTP 1991; Dietz et al. 1991
34	Mouse	(W)	13 wk		11298			NTP 1991; Dietz et al. 1991
Reproductive								
35	Rat	(W)	6 wk		1071			Larsen et al. 1991
36	Rat	(W)	13 wk		1700		3400 (increased testis weight, decreased sperm motility, caudal weight and epididymal weight, increased incidence of abnormal sperm)	NTP 1991; Dietz et al. 1991
					3100 (female)			
37	Mouse	(W)	13 wk		4858 (male) 11298 (female)			NTP 1991; Dietz et al. 1991

[a]The number corresponds to entries in Figure 2-2.
[b]Used to derive an intermediate oral minimal risk level (MRL) of 2 mg/kg/day; dose divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans, and 10 for human variability).

Cardio = cardiovascular; d = day(s); Derm/oc = dermal/ocular; (G) = gavage (vehicle not specified); (GW) = gavage water; (GO) = gavage oil; Gastro = gastrointestinal; Gd = gestation day(s); Gn pig = guinea pig; Hemato = hematological; LD_{50} = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; Musc/skel = muscular/skeletal; NOAEL = no-observed-adverse-effect level; Resp = respiratory; (W) = drinking water; wk = week(s); x = time(s)

FIGURE 2-2. Levels of Significant Exposure to Acetone - Oral

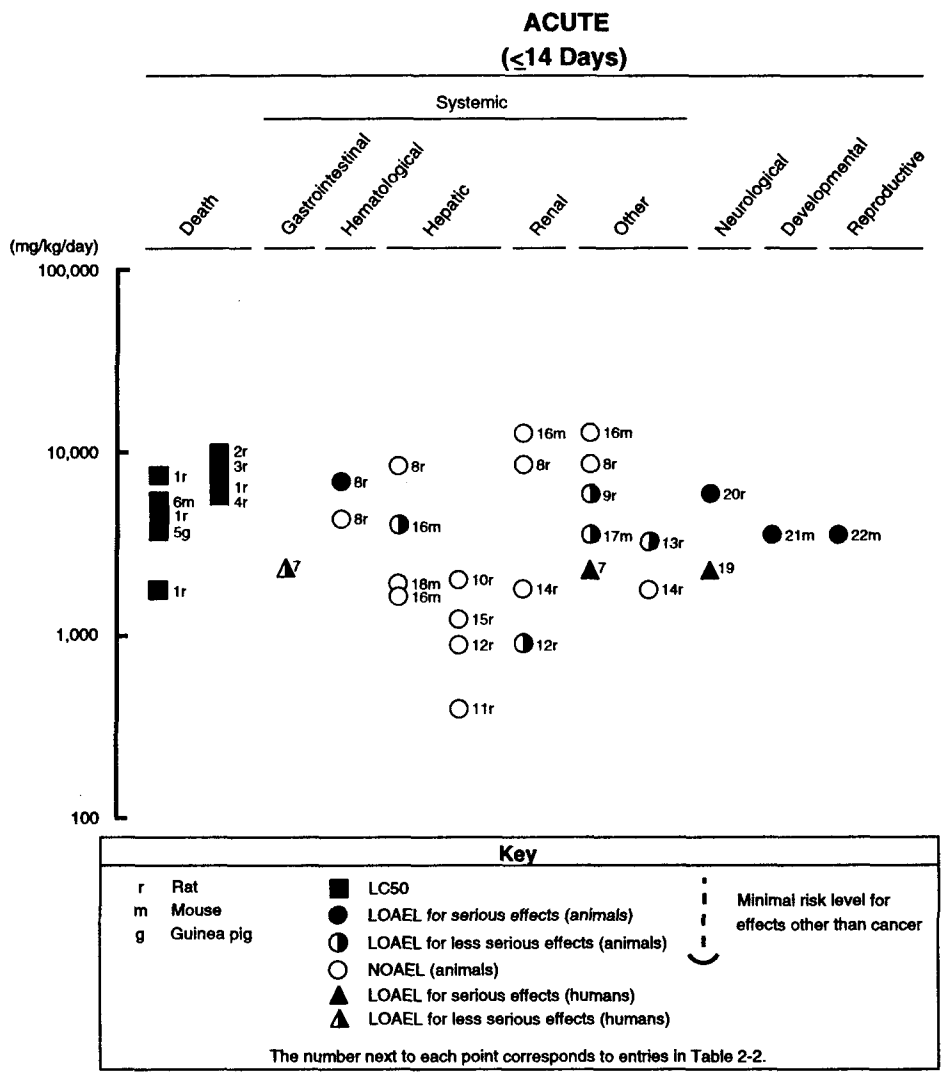

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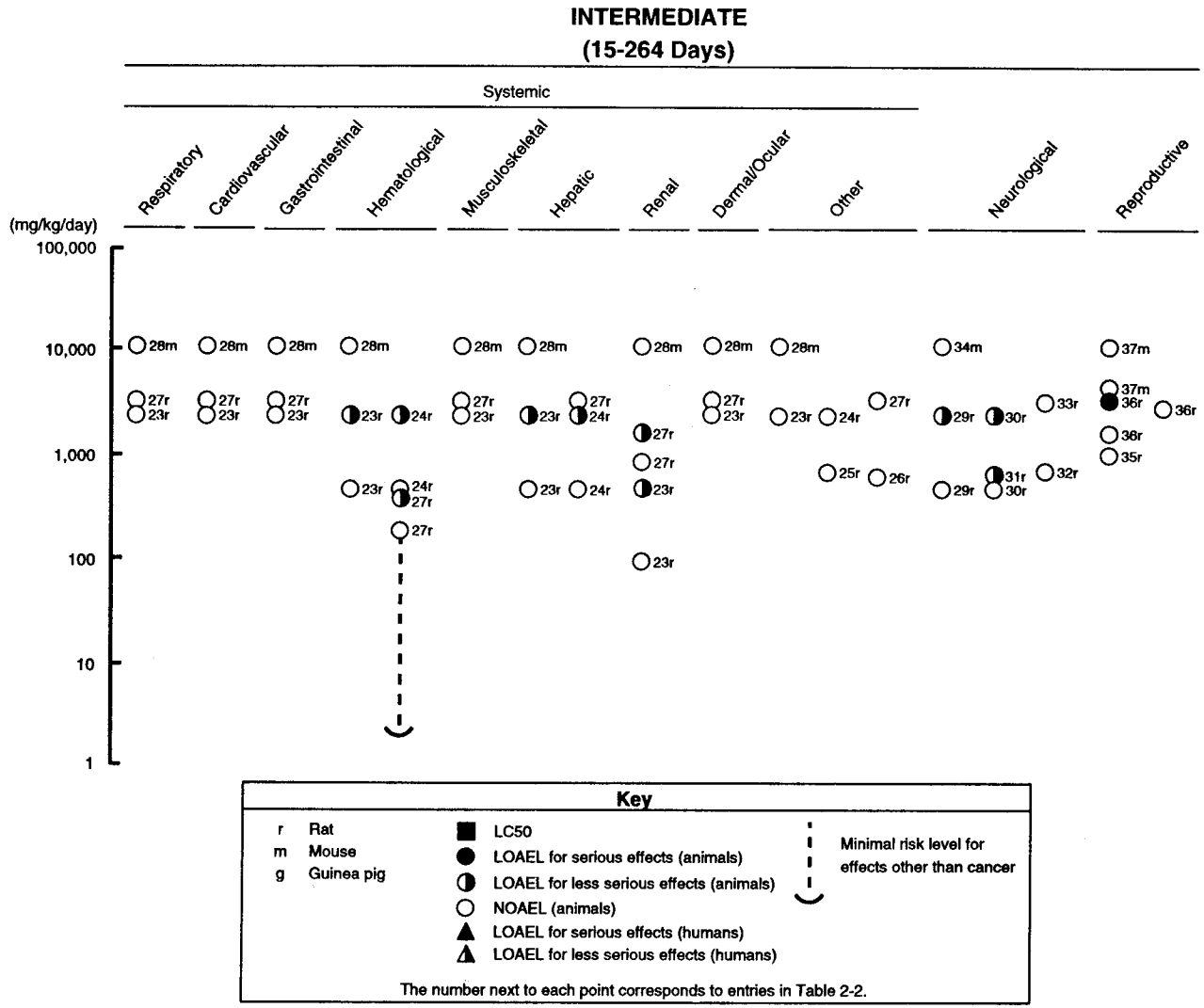
FIGURE 2-2. Levels of Significant Exposure to Acetone - Oral *(continued)*



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water for 7 days had a 500% increased activity of aniline hydroxylase activity, an activity associated with cytochrome P-450IIEl (Ueng et al. 1991). Furthermore, the level of cytochrome P-45011El and the activity of butanol oxidase increased 6-fold in microsomes from the nasal mucosa of rabbits exposed to acetone in drinking water for 1 week (Ding and Coon 1990). Induction of microsomal enzymes is considered a normal physiological response to xenobiotics, rather than an adverse effect, unless accompanied by increased organ weight and histopathological or other adverse respiratory effects. Changes in respiratory rates (either increases or decreases), along with signs of narcosis, were observed in rabbits dosed with ≥3,922 mg/kg acetone (Walton et al. 1928), and irregular respiration, along with signs of narcosis, was observed in dogs dosed with 4,000 mg/kg (Albertoni 1884). In a range-finding study to determine which doses to use in a developmental study, mice that died at doses ≥4,800 mg/kg/day for 10 days displayed wheezing and/or rapid and labored breathing, accompanied by signs of severe narcosis, prior to death (EHRT 1987). However, the apparent respiratory effects probably reflect the severely compromised condition of these animals, rather than a toxic effect of acetone on the lungs. Gross necropsy of a dog dosed with 8,000 mg/kg acetone revealed no effects on the lungs, but the lungs were not examined histologically (Albertoni 1884). Histological examination of the lungs of rats and mice exposed to acetone in drinking water for 13 weeks (Dietz et al. 1991; NTP 1991) or of rats given acetone in water by gavage for 13 weeks (American Biogenics Corp. 1986) revealed no treatment related lesions. Thus, acetone by itself apparently is not toxic to the lungs of animals when administered by the oral route, but the induction of lung microsomal enzymes suggests that acetone may potentiate the respiratory effects induced by other chemicals (see Section 2.6).

Cardiovascular Effects. Oral exposure of animals to acetone has not resulted in adverse effects on the heart in intermediate-duration studies. Histological examination of the hearts of rats and mice exposed to acetone in drinking water for 13 weeks (Dietz et al. 1991; NTP 1991) or of rats given acetone in water by gavage for 13 weeks (American Biogenics Corp. 1986) did not reveal treatment- related lesions. However, the heart-to-brain weight ratio was significantly increased ($p<0.01$) in the female rats treated by gavage with 2,500 mg/kg/day. In the absence of histologically observable lesions, the toxicological significance of the increased heart weight is questionable.

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects per se in humans after oral exposure to acetone, but a man who intentionally drank ≈200 mL of pure acetone

(≈2,241 mg/kg) had a red and swollen throat and erosions in the soft palate and entrance to the esophagus (Gitelson et al. 1966).

Significantly increased levels of cytochrome P-450IAl in duodenal microsomes and P-450IIB2 in duodenal and jejunal microsomes from rats exposed to acetone in drinking water for 3 days were found (Carriere et al. 1992). No increase in cytochrome P-450IIEl was found in these microsomal preparations. As discussed above for respiratory effects, induction of microsomal enzymes is considered a normal physiological response to xenobiotics rather than an adverse effect. Oral exposure of animals to acetone has not resulted in adverse effects on the gastrointestinal tract in intermediate-duration studies. Histological examination of the gastrointestinal tract of rats and mice exposed to acetone in drinking water for 13 weeks (Dietz et al. 1991; NTP 1991) or of rats given acetone in water by gavage for 13 weeks (American Biogenics Corp. 1986) did not reveal treatment-related lesions.

Hematological Effects. Exposure of rabbits to 863 mg/kg/day acetone in the drinking water for 7 days resulted in a 12.9-fold increase in the levels of cytochrome P-45011El in bone marrow microsomes (Schnier et al. 1989). As discussed for respiratory effects, induction of microsomal enzymes is considered a normal physiological response to xenobiotics rather than an adverse effect. Hematological effects of oral exposure to acetone have been observed in rats but not in mice. Bone marrow hypoplasia was observed in five of five male rats exposed to acetone in the drinking water for 14 days at 6,942 mg/kg/day, but not at 4,312 mg/kg/day (Dietz et al. 1991; NTP 1991). None of the female rats had bone marrow hypoplasia. Although mice were similarly treated for 14 days in this study, the authors did not specify whether bone marrow was examined; however, in the 13-week studies, no hematological effects or histologically observable lesions in hematopoietic tissues were found in mice. In contrast, evidence of macrocytic anemia was found in male rats exposed to acetone in drinking water for 13 weeks. This evidence consisted of significantly ($p<0.05$ or $p<0.01$) decreased hemoglobin concentration, increased mean corpuscular hemoglobin and mean corpuscular volume, decreased erythrocyte counts, decreased reticulocyte counts and platelets, and splenic hemosiderosis. The LOAEL for these effects was 400 mg/kg/day, and the NOAEL was 200 mg/kg/day. The number of affected parameters increased as the dose increased. Based on the NOAEL of 200 mg/kg/day for macrocytic anemia, an intermediate oral MRL of 2 mg/kg/day was calculated as described in the footnote in Table 2-2. In female rats, hematological effects consisted of statistically significant increased lymphocyte counts, increased mean corpuscular hemoglobin and mean corpuscular volume at the highest dose, and decreased platelets at the highest and next-to-highest dose levels (Dietz et al.

1991; NTP 1991). The biological significance of the hematological effects in female rats was not clear, but the effects were not consistent with anemia. Sex differences in the hematological effects of acetone exposure were also found in rats treated by gavage (American Biogenics Corp. 1986). Gavage treatment for 46-47 days significantly ($p<0.01$) increased hemoglobin, hematocrit, and mean cell volume in high-dose males (2,500 mg/kg/day), but not in females. With longer duration treatment (13 weeks), both high-dose males ($p<0.01$) and females ($p<0.05$) had increased hemoglobin and hematocrit, and high-dose males ($p<0.01$) also had increased mean cell hemoglobin and mean cell volume and decreased platelets. Thus, it appears that species and sex differences exist for hematological effects of oral exposure to acetone.

Musculoskeletal Effects. Histological examination of femurs of rats and mice exposed to acetone in drinking water for 13 weeks (Dietz et al. 1991; NTP 1991) or of rats given acetone in water by gavage for 13 weeks (American Biogenics Corp. 1986) did not reveal treatment-related lesions. Skeletal muscle was not examined histologically in the 13-week drinking water study (Dietz et al. 1991; NTP 1991), but histological examination of the skeletal muscle in rats in the 13-week gavage study did not reveal treatment-related lesions (American Biogenics Corp. 1986). Based on this limited information, it appears that acetone does not produce musculoskeletal effects.

Hepatic Effects. Acetone by itself is moderately toxic to the liver of animals, but acetone potentiates the hepatotoxicity of some other chemicals by inducing microsomal enzymes that metabolize other chemicals to reactive intermediates (see Sections 2.3.5 and 2.6). Numerous studies have investigated these mechanisms to identify the specific cytochrome P-450 isoenzymes involved (Banhegyi et al. 1988; Barnett et al. 1992; Carriere et al. 1992; Chieli et al. 1990; Furner et al. 1972; Gervasi et al. 1991; Hetu and Joly 1988; Hewitt et al. 1987; Hong et al. 1987; Hyland et al. 1992; Johannson et al. 1988; Kinsler et al. 1990; Kobusch et al. 1989; Koop et al. 1989, 1991; Menicagli et al. 1990; Porter et al. 1989; Puccini et al. 1989, 1990, 1992; Puntarulo and Cederbaum 1988; Robinson et al. 1989; Ronis et al. 1991; Ronis and Ingelman-Sundberg 1989; Schnier et al. 1989; Sipes et al. 1973; Song et al. 1989; Tu et al. 1983; Ueng et al. 1991; Yoo and Yang 1985). In these studies in general, rats, mice, rabbits, or hamsters were given acetone by gavage in water or in drinking water for 1 day to 2 weeks. Microsome preparations from the livers were then analyzed for cytochrome P-450 content, enzyme activities associated with specific cytochrome P-450 isoenzymes (particularly cytochrome P-450IIEl), and identification of the specific isoenzymes. Acetone has also been shown to increase the activity of glutathione S-transferase (Sippel et al. 1991). These topics are

discussed more fully in Sections 2.35 and 2.6. Induction of microsomal enzymes is considered a normal physiological response to xenobiotics rather than an adverse effect, unless it is accompanied by increased liver weight and other hepatic effects. Mice exposed to acetone in drinking water for 14 days had dose-related increased liver weights at ≥965 mg/kg/day, probably associated with microsomal enzyme induction (Dietz et al. 1991; NTP 1991). The increased liver weight was accompanied by hepatocellular hypertrophy at 23,896 mg/kg/day. In rats treated for 14 days, increased liver weight was stated to occur at the same or lower doses as in the 13-week study (see below), but more definitive information regarding the doses was not provided. Histological examination revealed no treatment-related hepatic effects in rats.

As stated above, acetone by itself is only moderately toxic to the liver. In mice exposed to 1,900 mg/kg/day acetone in the drinking water for 10 days, histological examination of the liver revealed no hepatic lesions (Jeffery et al. 1991). Acetone did not increase the level of serum alanine aminotransferase in rats at 871 mg/kg (Brown and Hewitt 1984), the levels of serum alanine aminotransferase or bilirubin at 1,177 mg/kg (Charbonneau et al. 1986b), or the activities of hepatic glucose-6-phosphatase, serum alanine aminotransferase, and serum ornithine carbamoyltransferase in rats given 1,961 mg/kg for 1 day or 392 mg/kg/day for 3 days (Plaa et al. 1982). However, in an intermediate-duration study, male rats, but not female rats, treated by gavage with 2,500 mg/kg/day, but not 500 mg/kg/day, for 46-47 days and for 13 weeks had statistically significant increased levels of serum alanine amino transferase (American Biogenics Corp. 1986). Liver weights were statistically significantly increased in female rats at ≥500 mg/kg/day, but not at 100 mg/kg/day, and in male rats at 2,500 mg/kg/day after 13 weeks, but organ weights were not measured in the rats treated for 46-47 days. In the 13-week drinking water study, liver weights were also significantly ($p<0.01$) increased in both sexes of rats at the same concentration (20,000 ppm, which was equivalent to 1,600 mg/kg/day for females, 1,700 mg/kg/day for males) and in female, but not male mice, at 11,298 mg/kg/day (Dietz et al. 1991; NTP 1991). However, in the mice, the increased liver weight was not associated with hepatocellular hypertrophy seen in the 14-day study, suggesting a development of tolerance.

Taken together, the data indicate that acetone induces liver microsomal enzymes, increases liver weights, and may cause liver injury, as evidenced by increased serum levels of liver enzymes associated with liver injury and hepatocellular hypertrophy. Species and sex differences exist in susceptibility to acetone-induced liver effects.

Renal Effects. Acetone can also induce enzymes in microsomes prepared from kidneys. In hamsters given drinking water containing acetone for 7 days (Ueng et al. 1991) or 10 days (Menicagli et al. 1990), the microsomes prepared from kidneys had increased levels of cytochrome P-450 and cytochrome b_5 and/or statistically significantly increased activities of p-nitrophenol hydroxylase, aniline hydroxylase, and aminopyrine-N-demethylase. Microsomes prepared from kidneys of rats treated by gavage with acetone had increased levels of cytochrome P-450IIEl and increased activity of N-nitrosodimethylamine demethylase (Hong et al. 1987). Induction of microsomal enzymes is considered to represent a normal physiological response to xenobiotics rather than an adverse effect, unless accompanied by increased organ weight and other adverse renal effects.

Oral exposure of rats and mice to acetone has resulted in effects on the kidney. Degeneration of the apical microvilli of renal tubules was reported in male rats after a single oral dose of acetone in corn oil, but not in corn oil treated controls (Brown and Hewitt 1984). The incidence of this lesion was not reported. However, in rats treated with 1,766 mg/kg/day acetone for 2 days, no significant difference was found for kidney weight, blood urea nitrogen (BUN) levels, or organic ion accumulation compared with controls (Valentovic et al. 1992). In 14-day drinking water studies, mice had doserelated increased kidney weights at $\geq$6,348 mg/kg/day (Dietz et al. 1991; NTP 1991). In rats treated for 16days, increased kidney weight occurred at the same or lower doses as in the 13-week study (see below), but more definitive information regarding the doses was not provided. Histological examination of the kidneys revealed no treatment-related lesions in rats or mice.

In the intermediate-duration drinking water study, significantly ($p<0.01$) increased kidney weights were seen in female rats at 21,600 mg/kg/day and in male rats at 3,400 mg/kg/day (Dietz et al. 1991; NTP 1991). Conversely, male, but not female rats, given acetone in the drinking water at 21,700 mg/kg/day had increased incidence and severity of nephropathy that was not accompanied by hyaline droplet accumulation (Dietz et al. 1991; NTP 1991). In the 13-week gavage study, kidney weights were significantly ($p<0.05$ or $p<0.01$) increased in female rats at $\geq$500 mg/kg/day and in male rats at 2,500 mg/kg/day (American Biogenics Corp. 1986). In addition, renal proximal tubule degeneration and intracytoplasmic droplets of granules (hyaline droplets) in the proximal tubular epithelium were seen in both control and treated rats at similar incidence, but the severity of these lesions showed a dose-related increase in males at $\geq$500 mg/kg/day and in females at 2,500 mg/kg/day. The renal lesions seen in both the gavage study and the drinking water study may represent an enhancement by acetone of the nephropathy commonly seen in aging rats (American Biogenics Corp.

1986; NTP 1991). No renal effects were observed in mice given acetone in the drinking water for 13 weeks (Dietz et al. 1991; NTP 1991).

Thus, species differences exist in susceptibility to acetone-induced renal effects. Sex differences also exist, with kidney weight increases occurring in female rats at lower doses than in males rats, but histopathological lesions occurring in male rats at lower doses than in females.

DermaVOcular Effects. No studies were located regarding dermal or ocular effects in humans after oral exposure to acetone.

Histological examination of eyes and skin of rats and mice after exposure to drinking water containing acetone for 13 weeks at doses ≤3,400 mg/kg/day (rats) and 11,298 mg/kg/day (mice) revealed no treatment-related effects (Dietz et al. 1991; NTP 1991). Similarly, ophthalmoscopic examination of the eyes of rats treated by gavage with acetone at doses ≤2,500 mg/kg/day revealed no ocular lesions (American Biogenics Corp. 1986). Skin was not examined histologically in the gavage study.

Other Systemic Effects. Acetone exposure of humans can result in diabetes-like symptoms, e.g., hyperglycemia and glycosuria. For example, a man who intentionally drank about 200 mL (about 2,241 mg/kg) of pure acetone had been treated at a hospital for acetone poisoning, but 4 weeks after the ingestion, he noticed excessive thirst and polyuria, and 2.5 months after ingestion, he was hyperglycemic (Gitelson et al. 1966). As discussed by Gitelson et al. (1966), hyperglycemia and glycosuria are commonly seen in cases of acetone poisoning.

Most of the information regarding other systemic effects in animals after oral exposure to acetone relates to body weight changes. However, in an acute study conducted to determine the temporal effects of maintaining elevated plasma concentrations of acetone similar to those encountered in fasting and diabetic patients, treatment of rats by gavage with 3,214 mg/kg/day resulted in significantly reduced ($p<0.01$) insulin-stimulated glucose oxidation in adipose tissue (Skutches et al. 1990). The reduction was greater in fasted rats than in fed rats. Rats treated by gavage with a lethal dose of acetone (LD_{50}= 5,800 mg/kg) lost 15% of their body weight until 48 hours after dosing (Freeman and Hayes 1985). However, treatment of rats by gavage with 1,766 mg/kg/day for 2 days (Valentovic et al. 1992) or with drinking water that provided lower doses (≤1,200 mg/kg/day) for up to 2 weeks (Furner et al. 1972; Hetu and Joly 1988) did not affect body weight gain. Rats maintained on drinking

water for 14 days at higher doses displayed decreased body weight gain >l0% of controls, but the decrease was associated with reduced water consumption probably due to unpalatability (Dietz et al. 1991; NTP). In contrast, mice similarly treated had decreased water consumption at doses ≥6,348 mg/kg/day, but no effects on body weight gain occurred at doses ≤12,725 mg/kg/day. Maternal body weight was slightly (5%) but significantly (p=0.02) reduced on day 3 postpartum in mice treated with 3,500 mg/kg/day acetone by gavage during gestation (EHRT 1987).

In intermediate-duration studies, gavage or drinking water treatment of rats or mice with acetone did not result in reductions in body weight except in cases where fluid consumption was reduced (American Biogenics Corp. 1986; Ladefoged et al. 1989; NTP 1991; Spencer et al. 1978).

2.2.2.3 Immunological Effects

No studies were located regarding immunological effects in humans or animals after oral exposure to acetone.

2.2.2.4 Neurological Effects

The narcotic effects of acetone occur after oral as well as inhalation exposure. Several case reports describe patients in minimally responsive, lethargic, or comatose conditions after ingesting acetone, but most of these cases are confounded by coexposure to other possible narcotic agents. For example, a 30-month-old child ingested most of a 6 ounce bottle of nail polish remover containing 65% acetone and 10% isopropyl alcohol (Gamis and Wasserman 1988); a known alcoholic woman ingested nail polish remover (Ramu et al. 1978); and a man ingested 200 mL of sake prior to intentionally ingesting liquid cement containing a mixture of polyvinyl chloride, acetone, 2-butanone, and cyclohexanone (Sakata et al. 1989). The lethargic and comatose condition of these patients were, however, attributed to acetone poisoning, although one case of coma was attributed primarily to cyclohexanol, the metabolite of cyclohexanone in the liquid cement (Sakata et al. 1989). Blood levels of acetone in some of these patients were 2.5 mg/mL (Ramu et al. 1978) and 4.45 mg/mL (Gamis and Wasserman 1988). In the case reported by Sakata et al. (1989), the blood level of acetone was 110 μg/L and the urine level was 123 μg/mL 5 hours after the ingestion, but the patient had been subjected to gastric lavage. A man who intentionally ingested about 200 mL of pure acetone (about 2,241 mg/kg) subsequently became deeply comatose, but responded to treatment (Gitelson et al. 1966). Six days

later, he was ambulatory, but a marked disturbance of gait was observed. This condition had improved upon follow-up examination 2 months later.

In acute experiments with animals, in which high oral doses of acetone resulted in death, severe neurological signs of toxicity preceded death. In a study to determine the LD_{50} value for acetone in rats (5,800 mg/kg), a state of prostration, usually without convulsions, preceded death (Freeman and Hayes 1985). In a study to determine which doses to use in a developmental study, oral dosing of pregnant mice with acetone during gestation resulted in languid behavior with subsequent death in one of four mice at 2,400 mg/kg/day (EHRT 1987). At higher doses, the number of mice dying increased, and they displayed a hunched appearance and became prostrate before death. No controls were used in this range-finding study. Rabbits dosed orally with 3,922, 5,491, or 7,844 acetone displayed signs of narcosis, the degree and the time to onset being dependent on dose (Walton et al. 1928). Signs of narcosis included weakness, depression, and unconsciousness. Puppies given 7,500 or 8,000 mg/kg and dogs given 4,000 mg/kg doses displayed incoordination, staggering, falling, tremors, delirium, prostration, and coma (Albertoni 1884). Dogs given 1,000 mg/kg showed no adverse effects. No controls were used in these studies, and only one or two animals were treated at each dose. A significant ($p<0.05$) reduction in nerve conduction velocity, but no effect on balance time in the rotorod test, was observed in rats treated for 6 weeks with acetone in drinking water at a dose of 650 mg/kg/day (Ladefoged et al. 1989). No reduction in nerve conduction velocity was found when tested at 3, 4, or 5 weeks of dosing. No histopathological lesions were found in tissues sampled from the cervico-medullary junction of the spinal cord; posterior tibia1 nerve proximal to the calf muscle branches; cerebellar vermis; thoracic, lumbar, and sacral spinal cord; L5 and L6 dorsal and ventral roots and spinal ganglia; and 3 levels of the sciatic nerve and the plantar nerves in the hindfeet of rats administered 732 mg/kg/day acetone in the drinking water for 12 weeks (Spencer et al. 1978). In another intermediate duration study, rats given 2,500 mg/kg/day acetone by gavage salivated excessively beginning on the 27th day of treatment (American Biogenics Corp. 1986). At the terminal sacrifice after 13 weeks of treatment, absolute brain weight was decreased in the male rats, but histological examination of the brain revealed no lesions. No clinical or histological evidence of neurotoxicity was observed in the rats or mice treated with higher doses for 13 weeks in the drinking water study (Dietz et al. 1991; NTP 1991). The fact that clinical signs of neurotoxicity were seen in the rats treated by gavage (American Biogenics Corp. 1986), but not in the rats or mice given higher doses in drinking water (NTP 1991), may reflect the intermittent nature of *ad libitum* dosing via drinking water, compared with the bolus nature of a gavage dose.

The highest NOAEL value and the LOAEL values for each species and duration category for neurological effects from all reliable studies are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to acetone. Reproductive effects were assessed in pregnant mice exposed by gavage to acetone during gestation (EHRT 1987). The reproductive index was significantly reduced ($p=0.05$) (number of females producing viable litters/number of surviving females that were ever pregnant; 24/31 treated compared with 34/36 controls). In addition, acetone treatment significantly ($p\leq0.01$) increased the duration of gestation from 18.1 days in controls to 18.5 days in treated mice.

No effects were observed on the fertility of male Wistar rats treated with drinking water containing acetone at 1,071 mg/kg/day for 6 weeks (Larsen et al. 1991). The indices of fertility examined were successful matings with untreated females, number of pregnancies, number of fetuses, testicular weight, seminiferous tubule diameter, and testicular lesions. However, male Sprague-Dawley rats treated with 3,400 mg/kg/day acetone in drinking water for 13 weeks had significantly increased ($p<0.01$) relative testis weight, probably because body weight was reduced, and significantly ($p<0.05$) decreased sperm motility, caudal weight and epididymal weight, and increased incidences of abnormal sperm (Dietz et al. 1991; NTP 1991). No testicular lesions were observed upon histological examination. Vaginal cytology examinations of the female rats revealed no effects. No effects on sperm morphology and vaginal cytology were observed in mice similarly treated with drinking water containing acetone at doses $\leq$4,858 mg/kg/day in males and $\leq$11,298 mg/kg/day in females.

The highest NOAEL values and the all LOAEL values in each species and duration category from all reliable studies are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans after oral exposure to acetone.

In a reproduction study, treatment of pregnant mice during gestation with acetone significantly ($\leq$10.01) reduced postnatal pup survival (EHRT 1987). The average weight of each live pup/litter was significantly reduced (p=0.0l) on postpartum day 0, but pups from the acetone treated groups gained significantly (p<0.0l) more weight than controls from postpartum day 0 to 3. As this study was not designed as a teratology study, fetuses or pups were not examined for internal malformations or skeletal anomalies.

The LOAEL value of 3,500 mg/kg/day for developmental effects in mice is recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or animals after oral exposure to acetone.

Genotoxicity studies are discussed in Section 2.4.

2.2.2.8 Cancer

No studies were located regarding cancer in humans or animals after oral exposure to acetone.

2.2.3 Dermal Exposure

2.2.3.1 Death

No studies were located regarding death of humans after dermal exposure to acetone.
In studies to determine the dermal LD_{50} values for acetone in rabbits (Roudabush et al. 1965; Smyth et al. 1962) and guinea pigs (Roudabush et al. 1965), the highest doses tested did not result in death. Therefore, LD_{50} values are >20 mL/kg (>15,688 mg/kg) for rabbits (Smyth et al. 1962) and >9.4 mL/kg (>7,373 mg/kg) for guinea pigs (Roudabush et al. 1965). No studies were located regarding death of animals after dermal exposure to acetone for intermediate- or chronic-durations.

2.2.3.2 Systemic Effects

No studies were located regarding respiratory, hematological, musculoskeletal, hepatic, or renal effects in humans after dermal exposure to acetone. Acetone has been used as a solvent or tested as a tumor promoter for other chemicals in skin painting studies in mice, and as the solvent control in these studies (Ward et al. 1986). An analysis of the histopathology in female SENCAR mice, used as acetone controls in a skin painting study of formaldehyde and held for up to 100 weeks of age, revealed no lesions associated with acetone exposure, that is, any lesions seen were considered spontaneous in this strain. Since all major tissues, gross lesions, and selected other (unspecified) tissues were examined histologically, it appears that no respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, or dermal/ocular lesions were observed upon histological examination. The mice had been treated with 0.2 mL acetone on their backs twice a week for 92 weeks. The dose of 0.2 mL is recorded as a NOAEL value in mice for each systemic effect for chronic-duration exposure in Table 2-3. No other studies were located regarding respiratory, gastrointestinal, hematological, or musculoskeletal effects in animals after dermal exposure to acetone. The cardiovascular, gastrointestinal, and dermal/ocular effects in humans and the cardiovascular, hepatic, renal, dermal/ocular, and other systemic effects in animals after dermal exposure to acetone in other studies are discussed below.

Cardiovascular Effects. As discussed in Section 2.2.1.2, high pulse rates (120-160/minute) were commonly found in patients exposed to acetone by inhalation and/or dermally after application of casts for which acetone was used in the setting solution (Chatterton and Elliott 1946; Hift and Pate1 1961; Pomerantz 1950; Renshaw and Mitchell 1956). Amyloidosis was observed in the hearts of mice whose lumbo-sacral regions were painted twice weekly with an unspecified quantity of acetone for 12 months (Barr-Nea and Wolman 1977).

Gastrointestinal Effects. As discussed in Section 2.2.1.2, case reports have described vomiting of blood and gastrointestinal hemorrhage in patients who had hip casts applied with acetone in the setting fluid (Chatterton and Elliott 1946; Fitzpatrick and Claire 1947; Harris and Jackson 1952; Hift and Pate1 1961; Pomerantz 1950; Renshaw and Mitchell 1956; Strong 1944). In most cases, exposure was considered to be mainly by inhalation, but dermal exposure could not be ruled out. In one case, the exposure was considered to be mainly dermal (Hift and Pate1 1961).

TABLE 2-3. Levels of Significant Exposure to Acetone - Dermal

Species	Exposure duration/ frequency	System	NOAEL	LOAEL (effect) Less serious	LOAEL (effect) Serious	Reference
ACUTE EXPOSURE						
Systemic						
Human	4-8 hr/d (occup)	Derm/oc		2000 ppm[a] (mild eye irritation)		Sallee and Sappington 1949
Human	2-3 d 8 hr/d	Derm/oc		901 ppm[a] (eye irritation)		Raleigh and McGee 1972
Human	1 d 2 min-4 hr/d	Derm/oc		12000 ppm[a] (eye irritation)		Ross 1973
Human	1 d 6 hr/d	Derm/oc		100 ppm[a] (eye irritation)		Matsushita et al. 1969b
Human	1 d 3-5 min/d	Derm/oc	200 ppm[a]	500 ppm[a] (eye irritation)		Nelson et al. 1943
Human	1 d 90 min/d	Derm/oc		1 mL (decreased protein synthesis)		Lupulescu and Birmingham 1975
Human	1 d 30 or 90 min/d	Derm/oc		1 mL (histological and ultrastructural degenerative changes in epidermis)		Lupulescu et al. 1972, 1973
Human	7 d 8 hr/d	Derm/oc		1006 ppm[a] (eye irritation)		Raleigh and McGee 1972
Rabbit	once	Derm/oc	0.01 mL			Smyth et al. 1962
Rabbit	once	Derm/oc			0.005 mL (severe eye necrosis)	Carpenter and Smyth 1941
Rabbit	once	Derm/oc			0.005 mL (severe corneal burn)	Smyth et al. 1962

TABLE 2-3. Levels of Significant Exposure to Acetone - Dermal *Continued)*

Species	Exposure duration/ frequency	System	NOAEL	LOAEL (effect) Less serious	LOAEL (effect) Serious	Reference
Rabbit	1 d 1 min/d	Derm/oc			20 (reversible corneal burns)	Bolkova and Cejkova 1983
Rabbit	1 d 3 min/d	Derm/oc		3.9 M (edema of eye mucous membrane)		Larson et al. 1956
Gn pig	1 d 25 min-23.4 hr/d	Derm/oc		21800 ppm[a] (lacrimation)		Specht et al. 1939
Mouse	once	Derm/oc		0.2 mL (slight increase in DNA synthesis in skin)		Iversen et al. 1988
INTERMEDIATE EXPOSURE						
Systemic						
Rabbit	3 wk 3 d/wk	Derm/oc	1.0 mL			Rengstorff et al. 1976
Rabbit	12-16 wk 1x/wk	Derm/oc		10 µL/wk (uveal melanocytic hyperplasia)		Pe'er et al. 1992
Gn pig	6 mo 5 d/wk 1x/d	Derm/oc Other		0.5 mL/d (mild erythema) 0.5 mL/d (transient weight loss of 60 g)		Taylor et al. 1993
Gn pig	3 wk 3 d/wk	Derm/oc			0.5mL (cataracts)	Rengstorff et al. 1972
Mouse	18 wk 2x/wk	Derm/oc		0.1 mL (moderate hyperplasia of epidermis)		Iversen et al. 1981

TABLE 2-3. Levels of Significant Exposure to Acetone - Dermal *Continued)*

Species	Exposure duration/ frequency	System	NOAEL	LOAEL (effect) Less serious	LOAEL (effect) Serious	Reference
CHRONIC EXPOSURE						
Systemic						
Mouse	92 wk 1x/wk	Resp	0.2 mL			Ward et al. 1986
		Cardio	0.2 mL			
		Gastro	0.2 mL			
		Hemato	0.2 mL			
		Musc/skel	0.2 mL			
		Hepatic	0.2 mL			
		Renal	0.2 mL			
		Derm/oc	0.2 mL			
		Other (all other tissues)	0.2 mL			
Mouse	502 d 3x/wk	Derm/oc		667 (dermatits in 2/40, hyperplasia in 1/40, and hyper-keratosis in 1/40)		DePass et al. 1989
Reproductive						
Mouse	92 wk 1x/wk		0.2 mL			Ward et al. 1986

[a]Exposure to acetone vapor in air

Cardio = cardiovascular; d = day(s); Derm/oc = dermal/ocular; DNA = deoxyribonucleic acid; Gastro = gastrointestinal; Gn pig = guinea pig; Hemato = hematological; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; min = minute(s); Musc/skel = muscular/skeletal; NOAEL = no-observed-adverse-effect level; occup = occupational; Resp = respiratory; wk = week(s); x = time(s)

Hepatic Effects. Amyloidosis was observed in the livers of mice whose lumbo-sacral regions were painted twice weekly with an unspecified quantity of acetone for 12 months (Barr-Nea and Wolman 1977).

Renal Effects. Amyloidosis was observed in the kidneys of mice whose lumbo-sacral regions were painted twice weekly with an unspecified quantity of acetone for 12 months (Barr-Nea and Wolman 1977).

Dermal/Ocular Effects. Liquid acetone has caused dermal effects in humans exposed by direct skin contact. Application of 1.0 mL directly to the skin of the forearms of six or seven volunteers for 30 or 90 minutes resulted in histological and ultrastructural degenerative changes in the epidermis (Lupulescu and Birmingham 1976; Lupulescu et al. 1972, 1973) and decreased protein synthesis (Lupulescu and Birmingham 1975) compared with untreated skin. The degenerative changes included a reduction and disorganization of the horny layers, intercellular edema, and vacuolization of the stratum spinosum. A laboratory technician being treated with squaric acid dibutyl ester in acetone for patchy alopecia areata on her scalp developed acute contact dermatitis after handling acetone for 2 years (Tosti et al. 1988). Patch testing with 10% acetone in olive oil showed a strong positive reaction (see Section 2.2.3.3).

Eye irritation is a common complaint of workers exposed to acetone vapors in the air at the workplace (Raleigh and McGee 1972) and in volunteers exposed to acetone in air under controlled laboratory conditions (Matsushita et al. 1969a, 1969b; Nelson et al. 1943). This eye irritation is due to direct contact of the eyes with the vapor rather than a systemic effect of vapor inhalation. Workers whose exposures to acetone in the workroom averaged ≥900 ppm complained of eye irritation (Raleigh and McGee 1972; Ross 1973). In controlled situations, the volunteers had been asked to give their subjective complaints, and some of the volunteers reported eye irritation after exposure to ≥100 ppm (Matsushita et al. 1969b). In a report of the experience at the Tennessee Eastman Corporation on acetone concentrations not associated with injury presented at the ACGIH Tenth Annual Meeting, it was noted that acetone is mildly irritating to the eyes at 2,000-3,000 ppm, with no irritation persisting after exposure ceases (Sallee and Sappington 1949).

Dermal effects have also been studied in animals after direct application of acetone to the skin. Application of 1.0 mL to the uncovered shaved skin of rabbits did not result in irritation within

24 hours (Smyth et al. 1962). Application of 0.2 mL acetone to the shaved skin of mice increased deoxyribonucleic acid (DNA) synthesis in the skin, compared to untreated shaved controls (Iversen et al. 1988). The increased DNA synthesis was considered a reaction to slight irritation. Moderate hyperplasia of the epidermis was observed in hairless mice treated twice weekly with 0.1 mL acetone for 18 weeks (Iversen et al. 1981). The hyperplasia persisted for 10 weeks after the end of treatment. Application of 0.5 mL/day for 6 months to the dorsal thorax of hairless guinea pigs resulted in only mild erythema at the site of application (Taylor et al. 1993). Amyloidosis was observed in the skin of mice whose lumbo-sacral regions were painted twice weekly with an unspecified quantity of acetone for 12 months (Barr-Nea and Wolman 1977). In a study in which acetone-treated mice were used as negative controls for a skin painting study of organosilanes, treatment with acetone alone 3 times/week for 502 days resulted in cases of hyperplasia (1 of 40), dermatitis (2 of 40), and hyperkeratosis (1 of 40) at the site of application (De Pass et al. 1989).

Ocular effects have been observed in animals after direct instillation of acetone into the eyes and after application of acetone to the skin. In rabbits, direct instillation of acetone into the eye has resulted in reversible cornea1 burns (Bolkova and Cejkova 1983), edema of mucous membranes (Larson et al. 1956), severe eye necrosis and cornea1 burns (Carpenter and Smyth 1946; Smyth et al. 1962), and uveal melanocytic hyperplasia (Pe’er et al. 1992). Application of 0.5 mL acetone directly to shaved skin of guinea pigs intermittently for 3 or 6 weeks resulted in cataract development (Rengstorff et al. 1972; Rengstorff and Khafagy 1985). In contrast, rabbits did not develop cataracts after application of 1.0 mL to the shaved skin intermittently for 3 weeks (Rengstorff et al. 1976). The difference in response between the guinea pigs and rabbits reflect species differences in susceptibility to the cataractogenic effects of acetone. Although the rabbits received twice as much acetone as the guinea pigs, the possibility that rabbits would have developed cataracts if an even larger quantity of acetone had been applied was not ruled out. However, no cataracts or lens opacities were found in hairless guinea pigs to which acetone (0.5 mL/day, 5 days/week) was applied to the skin for 6 months (Taylor et al. 1993). Genetic variability in susceptibility between the hairless guinea pigs (Taylor et al. 1993) and the normal guinea pigs (Rengstorff et al. 1972; Rengstorff and Khafagy 1985) is possible, but was believed to be unlikely (Taylor et al. 1993). Lacrimation was observed in guinea pigs exposed to acetone vapor in air at a concentration of 21,800 ppm for 25 minutes (Specht et al. 1939). The degree of lacrimation increased with longer exposure.

Other Systemic Effects. A transient weight loss of 60 g over a 2-week period was noted in hairless guinea pigs to which acetone was applied to the skin for 6 months (Taylor et al. 1993). Amyloidosis was observed in the adrenals and pancreas of mice whose lumbo-sacral regions were painted twice weekly with an unspecified quantity of acetone for 12 months (Barr-Nea and Wolman 1977).

2.2.3.3 Immunological Effects

The only information regarding immunological effects in humans after dermal exposure to acetone is a case report in which a laboratory technician being treated with squaric acid dibutyl ester in acetone for patchy alopecia areata on her scalp developed acute contact dermatitis after handling acetone for 2 years (Tosti et al. 1988). Patch testing with 10% acetone in olive oil showed a strong positive reaction. This acetone sensitization is considered a rare complication of sensitizing therapies for alopecia areata.

No studies were located regarding immunological effects in animals after dermal exposure to acetone.

2.2.3.4 Neurological Effects

As discussed in Section 2.2.1.4, case reports have described patients who became comatose or collapsed after hip casts were applied with acetone present in the setting fluid (Chatter-ton and Elliott 1946; Fitzpatrick and Claire 1947; Harris and Jackson 1952; Renshaw and Mitchell 1956; Strong 1944). In addition, a woman experienced headache, dizziness, weakness, difficulty speaking, and depression after a cast containing acetone had been applied (Pomerantz 1950). In another case of neurological effects (drowsiness, fretfulness, irritability, restlessness, uncoordinated hand movement, nystagmus) developing after application of a cast, exposure was considered to be mainly dermal because an airblower was used continuously during the application to dissipate the fumes (Hift and Pate1 1961). However, because the patient had kept his head under a blanket, some inhalation of acetone evaporating from cast may have occurred.

No studies were located regarding neurological effects in animals after dermal exposure to acetone.

2.2.3.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after dermal exposure to acetone.

Acetone has been used as a solvent or tested as a tumor promoter for other chemicals in skin painting studies in mice, and as the solvent control in these studies (Ward et al. 1986). An analysis of the histopathology in female SENCAR mice, used as acetone controls in a skin painting study of formaldehyde and held for up to 100 weeks of age, revealed no lesions associated with acetone exposure, that is, any lesions seen were considered spontaneous in this strain. Since all major tissues, gross lesions, and selected other (unspecified) tissues were examined histologically, it is assumed that no lesions in the female reproductive organs were observed upon histological examination. The mice had been treated with 0.2 mL acetone on their backs twice a week for 92 weeks. However, the dose of 0.2 mL cannot be considered a NOAEL for reproductive effects in the absence of data on male reproductive organs or reproductive studies in animals after dermal exposure to acetone.

2.2.3.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals after dermal exposure to acetone.

2.2.3.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or animals after dermal exposure to acetone.

Genotoxicity studies are discussed in Section 2.4.

2.2.3.8 Cancer

No studies were located regarding cancer in humans after dermal exposure to acetone.
Acetone has been used as a solvent for other chemicals in skin painting studies in mice, and as the solvent control in these studies (Ward et al. 1986). An analysis of the histopathology in female

SENCAR mice used as acetone controls in a skin painting study of formaldehyde and held for ≤100 weeks of age, revealed no neoplastic lesions associated with acetone exposure, that is, any lesions seen were considered spontaneous in this strain. Furthermore, acetone was negative as a tumor promoter for formaldehyde. In other skin painting studies in which acetone-treated mice were used as a negative vehicle control for organosilanes (De Pass et al. 1989) or flame retardants (Van Duuren et al. 1978), no evidence was found to suggest that acetone alone was a skin carcinogen. Acetone was also negative as a tumor initiator (Roe et al. 1972) and as a tumor promoter for 7,12-dimethylbenz[a]anthracene (Roe et al. 1972; Van Duuren et al. 1971; Weiss et al. 1986).

2.3 TOXICOKINETICS

Although the focus of this profile is on the effects of exposure to acetone from exogenous sources, a full understanding of the toxicokinetics requires consideration of the metabolic fate of endogenous acetone. Acetone is one of three ketone bodies that occurs naturally throughout the body (Le Baron 1982; Vance 1984). Under normal conditions, the production of ketone bodies occurs almost entirely within the liver and to a smaller extent in the lung and kidney (Gavin0 et al. 1987; Le Baron 1982; Vance 1984) The process is continuous, and the three products are excreted into the blood and transported to all tissues and organs of the body where they can be used as a source of energy. Two of these ketone bodies, acetoacetate and P-hydroxybutyrate, are organic acids that can cause metabolic acidosis when produced in large amounts. Acetone, in contrast, is nonionic and is derived endogenously from the spontaneous and enzymatic breakdown of acetoacetate (Kimura et al. 1986; Koorevaar and Van Stekelenburg 1976; Lopez-Soriano and Argiles 1985; Lopez-Soriano et al. 1985; Reichard et al. 1979; Van Stekelenburg and Koorevaar 1972). Endogenous acetone is eliminated from the body either by excretion into urine and exhaled air or by enzymatic metabolism (Charbonneau et al. 1986; Haggard et al. 1944; Owen et al. 1982; Reichard et al. 1986; Wigaeus et al. 1981). Under normal circumstances, metabolism is the predominant route of elimination and handles 70-80% of the total body burden.

Levels of endogenous acetone can fluctuate greatly due to normal diurnal variations (Wildenhoff 1972). In addition, circulating levels of endogenous acetone can fluctuate greatly depending on a person’s age (Paterson et al. 1967; Peden 1964), nutritional status and fasting (Jones 1987; Kundu et al. 1993; Levy et al. 1973; Lewis et al. 1977; Neiman et al. 1987; Reichard et al. 1979; Rooth and Carlstrom 1970; Williamson and Whitelaw 1978); and degree of physical activity (Koeslag et al.

1980). These physiological states all place high energy demands upon the body which result in increased fatty acid utilization and higher than normal blood levels of acetone. Infants and young children typically have higher acetone blood levels than adults due to their higher energy expenditure (Peden 1964). Pregnancy and lactation can also lead to higher than average blood levels of acetone (Bruss 1989; Paterson et al. 1967). In addition to these normal physiological conditions, a number of clinical states can result in humans acetonemia and acetonuria. In each of these conditions, the ketosis can be traced to the increased mobilization and utilization of free fatty acids by the liver. The conditions include diabetes (Kobayashi et al. 1983; Levey et al. 1964; Reichard et al. 1986; Rooth 1967; Rooth and Ostenson 1966), trauma (Smith et al. 1975), and alcoholism (Phillips et al. 1989; Tsukamoto et al. 1991).

Following exposure from exogenous sources, acetone is rapidly and passively absorbed from the lungs and gastrointestinal tract. Acetone can also be absorbed from the skin. After uptake by the lungs, acetone is readily absorbed into the bloodstream. Pulmonary uptake by humans has ranged from 30% to 80%. The reason for the wide range in reported values involves the unique aqueous wash-in/wash-out effect displayed when acetone is inhaled, causing spurious results. During this phenomenon, acetone, which is highly water soluble, will dissolve in epithelial cells during inspiration (wash-in) and evaporate during expiration (wash-out). This could account for lower than expected pulmonary absorption based on the high blood-air partition coefficient. Exhaled breath levels of acetone rise during exposure and reach steady state within 2 hours during exposure. Breath levels of acetone are directly proportional to the exposure concentration and duration and increase with physical activity due to increased pulmonary ventilation. Blood levels of acetone rose continuously during 2-4 hours exposure without reaching steady state, indicating continuous absorption during exposure. About 75-80% of the inspired amount is absorbed by blood within 15 minutes, and blood levels correlate with exposure concentration. Results in humans and animals indicate that relatively little acetone is absorbed from the nasal passages. No major differences were found between humans and animals in absorption after inhalation exposure. Based on animal studies, acetone is also absorbed rapidly and extensively from the gastrointestinal tract, with at least 74-83% absorbed based on the percent of the dose excreted by the lungs as acetone and carbon dioxide. As with inhalation, plasma levels rise proportionately with oral dose. Absorption can be delayed by the presence of fat in the stomach, due to delayed gastric emptying. Dermal absorption of acetone is also fairly rapid.

Acetone is highly water soluble and is widely distributed to tissues and organs throughout the body, especially to tissues with high water content. Radiolabeled unchanged acetone and total radioactivity (the percentage of total radioactivity that is not acetone represents metabolites) were detected in all tissues examined (blood, pancreas, spleen, thymus, heart, testis, vas deferens, lung, kidney, brain, liver, muscle, and adipose tissue) in male mice after inhalation exposure to radiolabeled acetone. Peak levels in these tissues occurred during the first 6 hours after exposure, and longer or repeated exposure resulted in no further accumulation except in liver and brown adipose tissue. Elimination of acetone from all tissues was complete within 24 hours, but total radioactivity, which represented metabolites, was still present in all tissues except blood and muscle. Thus acetone is not selectively distributed *to* any tissue and is not likely to accumulate with repeated exposure. Although information on distribution after oral and dermal exposure was not available, similar distribution is likely. Acetone can also undergo transplacental transfer to the fetus.

The metabolic fate of acetone, whether from endogenous or exogenous sources, is similar in humans and animals, is independent of route of exposure, and involves three separate gluconeogenic pathways, with ultimate incorporation of carbon atoms into glucose and other products of intermediary metabolism, with generation of carbon dioxide and adenosine triphosphate (ATP). Metabolism takes place primarily in the liver. Some of the exogenous acetone is unmetabolized and excreted primarily in the expired air. Acetone is initially oxidized to acetol by acetone monooxygenase, the rate limiting step governing the overall accumulation and elimination of acetone from the body. Acetol is oxidized to methylglyoxal by acetol monooxygenase. Both activities are associated with P-450IIE1, and the reactions require oxygen and nicotinamide adenine dinucleotide phosphate (NADPH). Methylglyoxal is converted to glucose directly or via D-lactate. Acetol can also be converted to L-1,2-propanediol, from which the pathways branch with either formation of L-lactate to D-glucose or degradation of 1,2-propanediol to acetate and formate. The carbon atoms of acetone can also be incorporated into glycogen, amino acids, fatty acid, heme, cholesterol, choline, and urea via acetate and formate. These pathways appear to operate in rats, mice, and rabbits, with very few species differences, and hence probably in humans. The relative importance of the three pathways depends upon the dose, with the methylglyoxal and lactate pathways predominating at low doses, but shunting to the formate-acetate branch of the propanediol pathway at higher doses as the methylglyoxal and lactate pathways become saturated. Physiological status, such as diabetes and fasting, and genetic status can alter the pattern of metabolism.

The main route of excretion is via the lungs regardless of the route of exposure with very little excreted in the urine. Acetone is excreted both unchanged and, following metabolism, mainly as carbon dioxide. In humans exposed by inhalation, the rate and pattern of respiratory and urinary excretion of acetone is influenced by exposure concentration, duration, the level of physical activity during exposure, and gender. Respiratory excretion is complete within 20 hours after inhalation, and peak urinary excretion occurs between 1 and 3.5 hours after exposure. The amount expired or excreted in the urine increases with increasing exposure concentration and with increasing duration of exposure, and increases with exercise during exposure. Women expired acetone more slowly than men, but the percentages excreted were not significantly different. The time for urinary excretion to be complete increases with exposure concentration and duration. Daily intermittent exposure may result in a slight residual body burden, but elimination is generally complete in 48-72 hours after the last exposure, depending on the exposure concentration. Excretion of acetone after inhalation exposure is better characterized in humans than in animals, but appears to be similar. In rats exposed to radiolabeled acetone, 52% of the expired radioactivity was unchanged acetone and 48% was carbon dioxide. Based on animal studies, excretion of acetone and carbon dioxide after oral exposure is similar to that after inhalation exposure.

Most of the toxic effects of acetone do not appear to be due to any of its metabolites. As is typical of solvents, acetone is irritating to the mucous membranes. Acetone is also narcotic, and although the mechanism by which acetone exerts is effects on the central nervous system is unknown, as a solvent, it may interfere with the composition of membranes, altering their permeability to ions. The mechanisms by which acetone produces hematological, hepatic, renal, reproductive, and developmental effects is unknown, but acetone has been found to distribute to all of these target organs, including the brain, and can undergo transplacental transfer. The renal toxicity may be due to the formation of formate and may involve α_{2u}-globulin. One of the main effects of acetone is the induction of microsomal enzymes, particularly cytochrome P-450IIEl. Enzyme induction is probably responsible for the increased liver and kidney weights observed in animals by virtue of the increase in protein content. Acetone also potentiates the toxicity of numerous other chemicals primarily by increasing their metabolism to toxic intermediates by the induction of cytochrome P-450IIE1, or otherwise interfering with their metabolism and elimination.

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

Due to its high blood-air partition coefficient (167-330) (Fiserova-Bergerova and Diaz 1986; Haggard et al. 1944; Paterson and Mackay 1989; Sato and Nakajima 1979), acetone is rapidly and passively taken up by the respiratory tract and absorbed into the bloodstream during inhalation exposure. Experiments in humans exposed to 23-4,607 ppm for up to 4 hours have measured pulmonary uptakes ranging from ≈30% to 80% (DiVincenzo et al. 1973; Landahl and Herrmann 1950; Nomiyama and Nomiyama 1974a; Pezzagno et al. 1986; Wigaeus et al. 1981). The reason for the wide range in reported values involves the unique aqueous wash-in/wash-out effect when acetone in inhaled, which can lead to spurious results (Schrikker et al. 1985, 1989). During this phenomenon, acetone, which is highly water soluble, will dissolve in epithelial cells during inspiration (wash-in) and evaporate during expiration (wash-out). This could account for the lower than expected pulmonary absorption based on the high blood/air partition coefficient (Wigaeus et al. 1981). Exhaled breath levels of acetone in humans rose during exposure and reached steady-state within ≈2 hours during exposure (Brown et al. 1987; DiVincenzo et al. 1973; Nomiyama and Nomiyama 1974a). Uptake was directly proportional to exposure concentration and duration (DiVincenzo et al. 1973; Wigaeus et al. 1981). Uptake also increased as the level of physical activity increased, i.e., during exercise, due to increased pulmonary ventilation (DiVincenzo et al. 1973; Haggard et al. 1944; Jakubowski and Wieczorek 1988; Wigaeus et al. 1981). Lungs (including the mouth and trachea) retained a greater percentage of inspired acetone (55%) than the nasal cavity (18%) in humans, indicating that the nasal cavity absorbs acetone less readily than the rest of the respiratory system (Landahl and Herrmann 1950). Blood levels of acetone rose rapidly during exposure for up to 4 hours with no indication that steady-state was reached (Brown et al. 1987; Dick et al. 1989; DiVincenzo et al. 1973), suggesting that during exposure, the rate of absorption exceeded the rate of distribution and elimination. In humans exposed to 100 or 500 ppm acetone for 2 or 4 hours, 75-80% of the amount of acetone inspired was absorbed by blood after 15 minutes of exposure, and 20-25% remained in the dead space volume (DiVincenzo et al. 1973). Higher inspired amounts resulted in higher blood levels (DiVincenzo et al. 1973; Haggard et al. 1944; Matsushita et al. 1969a; Pezzagno et al. 1986). A correlation between blood level at the end of exposure and exposure concentration was found in humans exposed to 23-208 ppm for 2-4 hours (Pezzagno et al. 1986). No significant difference in uptake or retention was found between men and women (Brown et al. 1987).

Animals also absorb acetone rapidly during inhalation exposure. Measurement of blood acetone levels in rats after 4-6 hours of exposure to various concentrations shows that blood levels correlate well with exposure concentrations (Charbonneau et al. 1986a, 1991; NTP 1988) and are highest immediately after exposure (NTP 1988). In rats exposed to 1.50 ppm for 0.5-4 hours, measurement of blood acetone concentrations during exposure revealed that blood levels increased steadily for 2 hours and then remained constant for the next 2 hours of exposure (Geller et al. 1979b). Blood acetone levels also correlated well with exposure concentration in dogs exposed for 2 hours (DiVincenzo et al. 1973). Blood levels were 4, 12, and 25 mg/L after exposures to 100, 500, and 1,000 ppm, respectively. Comparison of uptakes in dogs and humans revealed that humans absorbed a greater absolute quantity under comparable exposure conditions, but when expressed in terms of kg body weight, dogs absorbed 5 times more than humans. In anesthetized dogs allowed to inhale concentrated vapors of acetone spontaneously from a respirator at various ventilation rates, uptake by the respiratory tract was 52% at flow rates of 5-18 L/minute and 42% at ventilation rates of 21-44 L/minute (Egle 1973). Retention in the lower respiratory tract was 48% at 5-18 L/minute and 37.5% at 2140 L/minute. Retention by the upper respiratory tract was 57% at 4-18 L/minute. The effect of exposure concentration on total uptake was studied at a range of ventilation rates equated with exposure concentrations. Percent uptakes were 52.1% at a mean concentration of 212 ppm, 52.9% at 283 ppm, and 58.7% at 654 ppm. These results indicate the respiratory uptake of acetone by dogs is similar to human uptake values reported by Landahl and Herrmann (1950). The retention in the upper respiratory tract was higher than in the lower respiratory tract of dogs (Egle 1973). Exposure concentration had little effect on retention. The absorption of acetone by the nasal walls of anesthetized dogs, in which the nasal passage was isolated, increased when the airflow rate was increased (Aharonson et al. 1974). This suggests that increased airflow decreases the amount of acetone that reaches the lungs.

In rats exposed continuously to 2,210 ppm for 9 days, peak acetone blood levels of ≈ 1,020-l ,050 mg/L were reached in 3-4 days and remained at this level for the duration of exposure (Haggard et al. 1944) In rats exposed to 4,294 ppm for 12 days, acetone blood levels plateaued at 2,420-2,500 mg/L in 4 days. Blood levels in rats exposed to these concentrations for 8 hours/day were about half of those reached during continuous exposure. The amount of acetone absorbed in the first 8 hours exceeded the amount eliminated in the next 16 hours of exposure to fresh air, leading to a small accumulation. However, the accumulation during intermittent exposure did not reach the levels

achieved during continuous exposure. In other experiments of rats exposed to 2,105-126,291 ppm, the time to peak blood level decreased as the exposure concentration increased.

As was found in humans (Landahl and Herrmann 1950) and dogs (Egle 1973), disposition of acetone in the upper respiratory tract of rats, mice, guinea pigs, and hamsters indicates that relatively little acetone is absorbed from the upper respiratory tract (Morris 1991; Morris and Cavanagh 1986, 1987; Morris et al. 1986, 1991). The deposition efficiency was greater in Sprague-Dawley rats than in Fischer-344 rats. Deposition was similar in B6C3Fl mice and Fischer-344 rats, and greater than in Hartley guinea pigs and Syrian golden hamster. No difference was found between male and female Sprague-Dawley rats (Morris et al. 1991). The differences among strains and species could not be attributed to differences in metabolism because acetone is not significantly metabolized in the upper respiratory tract of these species (Morris 1991). Rather, the difference was attributed to differences in perfusion rates (Morris 1991; Morris and Cavanagh 1987).

2.3.1.2 Oral Exposure

In a series of experiments conducted in male volunteers given acetone orally at 40-80 mg/kg, an estimated 65-93% of the administered dose was eliminated via metabolism, with the remainder excreted in the urine and expired air in about 2 hours, indicating rapid and extensive gastrointestinal absorption (Haggard et al. 1944). In a human who ingested 137 mg/kg acetone on an empty stomach, the blood level of acetone rose sharply to a peak 10 minutes after dosing (Widmark 1919). In other experiments, the subject ingested the same dose 10 or 12 minutes after eating porridge. The blood acetone level rose slowly over 48-59 minutes to levels of about one-half to two-thirds that achieved after taking acetone on an empty stomach. Thus, the presence of food in the gastrointestinal tract lead to a slower rate of absorption.

Measurement of acetone in blood and urine of patients who accidentally or intentionally ingested acetone indicated that acetone was absorbed, but the percentage absorbed cannot be determined from the data. In one case, a man ingested liquid cement that provided a dose of acetone of ≈231 mg/kg (Sakata et al. 1989). His plasma acetone level was about 110 μg/mL and his urinary level was 123 μg/mL 5 hours after ingestion, but he had been subjected to gastric lavage. In a another case, a woman who had ingested nail polish remover had a blood acetone level of 0.25 g/100 mL (2.5 mg/mL) upon admission to the hospital (Ramu et al. 1978). The authors estimated that her body

burden was 150 g acetone at the time of admission. The serum acetone level of a 30-month-old child was 445 mg/l00 mL (4.45 mg/rnL) 1 hour after ingestion of a 6ounce bottle of nail polish remover (65% acetone) (Gamis and Wasserman 1988).

Experiments in rats indicated that acetone is rapidly and almost completely absorbed from the gastrointestinal tract after oral exposure. A rat given ^{14}C-acetone at a dose of 1.16 mg/kg expired 47.4% of the dose as ^{14}C-carbon dioxide over the 13.5-hour collection period (Price and Rittenberg 1950). Another rat given about 7.11 mg/kg ^{14}C-acetone by gavage once a day for 7 days expired 67-76% of the administered radioactivity as ^{14}C-carbon dioxide and 7% as ^{14}C-acetone over a 24-hour period after the last dose. From these data, absorption of least 74-83% of the administered dose can be inferred. A rat dosed with 6.19 mg/kg ^{14}C-acetone expired 4.24% of the radiolabel as unchanged ^{14}C-acetone over 5.5 hours, indicating rapid absorption. In rats given a single gavage dose of 1,177 mg/kg acetone, the maximum blood level of 850 μg/mL was reached in 1 hour and declined gradually to about 10 μg /mL over 30 hours (Plaa et al. 1982). In another experiment, peak blood levels and the time to peak blood levels were compared after various gavage doses to rats. After a dose of 78.44 mg/kg, the maximum blood level of acetone of about 200 μg/mL was reached in 3 hours and declined to 10 μg/mL at 12 hours, where it remained for the next 12 hours. After a dose of 196.1 mg/kg, the peak blood level was 400 μg/mL at 6 hours and declined biphasically to 50 μg/mL at 12 hours and to 30 μg/mL at 18 hours where it remained for the next 6 hours. After a dose of 784.4 mg/kg, the peak level was 900 μg/mL at 1 hour and declined to 300 μg /mL at 12 hours, 110 μg/mL at 18 hours, and 50 μg/mL at 24 hours. After a dose of 1,961 mg/kg, the peak level was 1,900 μg /mL at 3 hours and declined slowly to 400 μg /mL at 24 hours. In other studies where rats were given similar or higher doses of acetone, plasma acetone levels rose proportionately with dose in rats given acetone as single doses by gavage (Charbonneau et al. 1986a; Lewis et al. 1984) or in the drinking water for 7 days (Skutches et al. 1990).

In a study comparing the blood levels of acetone achieved after fasting with those after oral dosing, peak blood levels of acetone of about 35 and 110 μg /mL were reached within about 3 hours after dosing of rats with 78 and 196 mg/kg acetone, respectively (Miller and Yang 1984). The levels declined to near background levels within the next 16 hours. At an acetone dose of 20 mg/kg, the blood level increased to about 5 μg /mL over 19 hours, when the rats were sacrificed. In rats fasted for 48 hours, blood acetone levels increased continuously to about 13 μg /mL. While the maximal blood

concentrations of the treated rats differed considerably from that of the fasting group, the areas under the curve for the 78 and 196 mg/kg groups were comparable to the fasting groups.

Conflicting data were located regarding the effect of vehicle on the gastrointestinal absorption of acetone. In one study, maximum blood levels were higher and achieved earlier in rats given acetone by gavage in water than in rats given acetone by gavage in corn oil (Charbonneau et al. 1986a). The slower absorption of acetone in corn oil may have resulted from a delayed gastric emptying due to the presence of corn oil (fat) in the stomach. In a later study, however, very little difference in blood and liver levels of acetone were found in rats given the same dose of acetone in water or in corn oil (Charbonneau et al. 1991).

No studies were located regarding absorption of acetone in other animal species after oral exposure to acetone.

2.3.1.3 Dermal Exposure

Dermal absorption of acetone has been demonstrated in humans. Application of cotton soaked in acetone to a 12.5 cm^2 uncovered area of skin of volunteers for 2 hours/day for 4 days resulted in blood levels of acetone of 5-12 μg /mL, alveolar air levels of 5-12 ppm, and urinary concentrations of 8-14 μg /mL on each day (Fukabori et al. 1979). Higher blood, alveolar air, and urinary levels were obtained when the daily exposure increased to 4 hour/day: 26-44 μg /mL in blood, 25-34 ppm in alveolar air, and 29-41 μg /mL in urine. The absorption was fairly rapid, with peak blood levels appearing at the end of each daily application. Although precautions were taken to limit inhalation of acetone vapors, the authors noted that it was not possible to completely prevent inhalation, and the acetone concentration in the breathing zone of one subject was found to be 0.4-0.6 ppm. From the alveolar air and urine concentrations, it was estimated that a 2-hour dermal exposure was equivalent to a 2-hour inhalation exposure to 50-150 ppm, and a 4-hour dermal exposure was equivalent to a 2-hour inhalation exposure to 250-500 ppm acetone.

No studies were located regarding the absorption of acetone in animals after dermal exposure. The findings of cataract formation in guinea pigs exposed dermally (Rengstorff et al. 1972; Rengstorff and Khafagy 1985) (see Section 2.2.3.2) however, indicated that acetone was absorbed from the skin of the guinea pigs.

2.3.2 Distribution

Of eight samples of breast milk from lactating women from four urban areas, all were found to contain acetone (Pellizzari et al. 1982). Whether the source of acetone was endogenous or exogenous could not be determined. Nevertheless, the data indicate that acetone is distributed to mother's milk, and represents a source of excretion from the mother and exposure for infants. Acetone was identified in maternal and cord blood collected at the time of delivery, indicating transplacental transfer (Dowty et al. 1976).

2.3.2.1 Inhalation Exposure

No studies were located regarding distribution of acetone or its metabolites in humans after inhalation exposure to acetone. However, acetone is well absorbed into the blood from the respiratory tract of humans (see Section 2.3.1 .l) and is highly water soluble. Therefore, widespread distribution, especially to tissues with high water content, is expected.

The distribution of acetone has been studied in mice exposed to acetone by inhalation (Wigaeus et al. 1982). Mice were exposed to 500 ppm ^{14}C-acetone for 1, 3, 6, 12, and 24 hours or for 6 hours/day for 1, 3, or 5 consecutive days, after which they were immediately killed. Radioactive unmetabolized acetone and total radioactivity were found in blood, pancreas, spleen, thymus, heart, testis, vas deferens, lung, kidney, brain, liver, muscle, brown adipose tissue, subcutaneous adipose tissue, and intraperitoneal adipose tissue. A common feature was an increase in tissue concentration of acetone and total radioactivity during the first 6 hours after exposure. These levels generally peaked from about 2.5 to 3.5 μmol/g tissue except in adipose tissues for total radioactivity. Peak levels of unmetabolized acetone were generally <l-l.3 μmol/g tissue. Exposure for longer than 6 hours resulted in no further accumulation of total radioactivity except in the liver and brown adipose tissue, in which levels rose to 4.8 μmol /g liver and 2.6 μmol /g brown adipose tissue at 24 hours. Only about 10% of the radioactivity in the liver at 24 hours was unmetabolized acetone. When the mice were exposed intermittently on 3 or 5 consecutive days, most tissues showed no or only a small additional increase in radioactivity after more than 1 day of exposure; however, the concentration in adipose tissue increased significantly with increasing exposure duration ≤5 days. The ratio of acetone in the tissues to that in blood was <1 at all exposure times except for the lungs (the site of exposure). However, the ratio of total radioactivity in the tissues to that in the blood showed that after 1 and

3 hours exposure, only the lung had a ratio >l, whereas the ratios in the kidneys and liver were >1 after 6 hours. Only the muscle and subcutaneous and intraperitoneal adipose tissue rose continuously. Elimination of acetone was fastest in blood, kidneys, lungs, brain, and muscles with half-times of about 2-3 hours during the first 6 hours after exposure. The slowest elimination was in subcutaneous adipose tissue with a half-time of >5 hours. Elimination of acetone was complete in all tissues by 24 hours after exposure, but total radioactivity, indicative of metabolites, was still present in all tissues except blood and muscle. These data indicate that acetone is not selectively distributed to any tissues but is more evenly distributed in body water. Acetone is not likely to accumulate with repeated exposure. The continued accumulation of radioactivity in the liver and brown adipose tissue could be the result of high metabolic turnover in these tissues.

2.3.2.2 Oral Exposure

No studies were located regarding the distribution of acetone or its metabolites in humans or animals after oral exposure except that acetone was found in the liver of rats after oral exposure (Charbonneau et al. 1986a, 1991). However, acetone is well absorbed from the gastrointestinal tract (see Section 2.2.1.2) and is highly water soluble. Therefore, widespread distribution, especially to tissues with high water content, is expected.

2.3.2.3 Dermal Exposure

No studies were located regarding distribution of acetone or its metabolites in humans or animals after dermal exposure. The findings of cataract formation in guinea pigs exposed dermally (Rengstorff et al. 1972; Rengstorff and Khafagy 1985) (see Section 2.2.3.2), however, indicated that acetone was absorbed from the skin of the guinea pigs and distributed to the eyes.

2.3.2.4 Other Routes of Exposure

The finding of acetone and its metabolites in fetuses from rats injected intravenously with 100 mg/kg acetone on gestational day 19 indicates transplacental transfer (Peinado et al. 1986).

2.3.3 Metabolism

The metabolic fate of acetone is independent of route of administration and involves three separate gluconeogenic pathways, with ultimate incorporation of carbon atoms into glucose and other products and substrates of intermediary metabolism with generation of carbon dioxide. The metabolic pathways appear to be similar in humans and animals. The primary (major) pathway involves hepatic metabolism of acetone to acetol and hepatic metabolism of acetol to methylglyoxal, while two secondary (minor) pathways are partially extrahepatic, involving the extrahepatic reduction of acetol to L-1,2-propanediol. Some of exogenous acetone is unmetabolized and is excreted primarily in the expired air with little acetone excreted in urine (see Section 2.3.4).

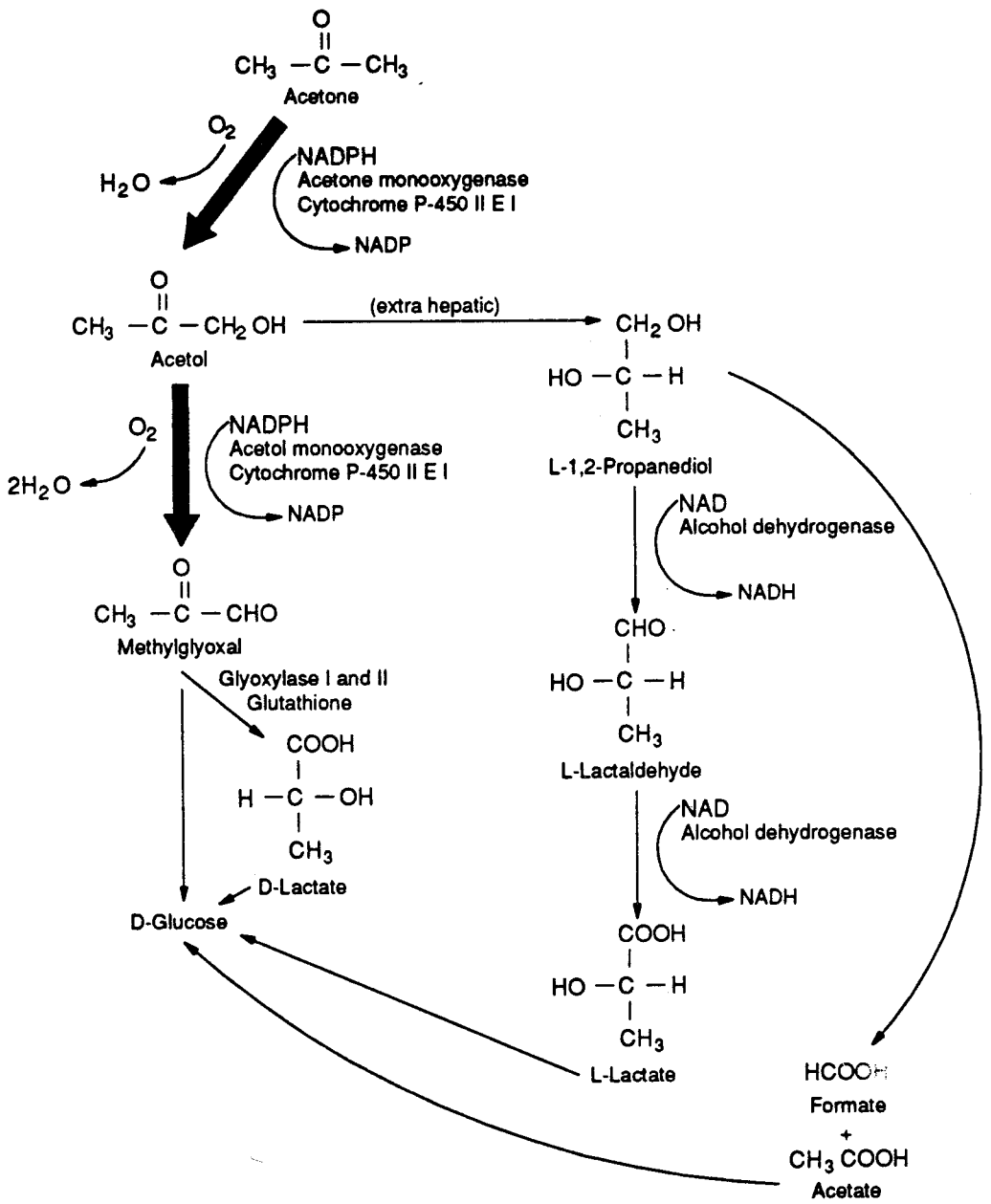
The only studies located regarding the metabolism of acetone in humans were conducted in normal fasted, obese fasted, and diabetic patients (Reichard et al. 1979; 1986). The involvement of gluconeogenesis was demonstrated in normal patients fasted for 3 days, obese patients fasted for 3 days, and obese patients fasted for 21 days before intravenous injection of 2-[^{14}C]-acetone (Reichard et al. 1979). The percentages of ^{14}C-glucose in plasma derived from ^{14}C-acetone were 4.2%, 3.1%, and 11.0% in the three respective groups, suggesting the involvement of gluconeogenesis. Cumulative ^{14}C-carbon dioxide excretion by the lungs during the a 6-hour collection period accounted for 17.4%, 21.5%, and 4.9% in the three respective groups. Radioactivity was also incorporated into plasma lipids and plasma proteins. Unmetabolized acetone in the expired air accounted for 14.7%, 5.3%, and 25.2%, urinary excretion of acetone accounted for 1.4%, 0.6%, and 1.3%, respectively, and *in vivo* metabolism accounted for 83.%, 94.1%, and 73.%, respectively, of the radioactivity. Intravenous infusion of 2-[^{14}C]-acetone into patients with diabetic ketoacidosis resulted in a mean plasma acetone turnover rate of 6.45 μmol /kg/minute (Reichard et al. 1986). Analysis of glucose in urine revealed a labeling pattern in five of the six patients consistent with the involvement of pyruvate in the gluconeogenic pathway. A different pathway may have operated in the other patient. Acetol and 1,2-propanediol were also detected in the plasma and the concentrations of these metabolites were directly related to the plasma level of acetone. The results demonstrated high plasma acetone levels in decompensated diabetic patients. The suggested pathway of acetone metabolism in these patients was acetone to acetol to 1,2-propanediol to pyruvate and ultimately to glucose, but other pathways may exist between subclasses of diabetic patients.

The metabolism of acetone has been studied extensively in animals, primarily in rats, and three separate pathways of gluconeogenesis have been elucidated (see Figure 2-3). These pathways are consistent with the metabolic fate of acetone in humans, discussed above. The elucidation of these pathways has been performed in experiments in which rats, mice, or rabbits were exposed by inhalation, by gavage, via drinking water, or by intravenous, subcutaneous, or intraperitoneal injection of nonradiolabeled acetone or to acetone labeled with ^{14}C in the methyl groups, number 2 carbon atom, or all three carbon atoms (Casazza et al. 1984; HaIlier et al. 1981; Hetenyi and Ferrarotto 1985; Johansson et al. 1986; Koop and Casazza 1985; Kosugi et al. 1986a, 1986b; Mourkides et al. 1959; Price and Rittenberg 1950; Puccini et al. 1990; Rudney 1954; Sakami and LaFaye 1950, 1951; Skutches et al. 1990). In these experiments, identification of metabolites in liver, plasma, or urine, the labeling patterns of ^{14}C incorporation into metabolites from ^{14}C-acetone in plasma or in liver, or the results of enzyme reactions using microsomes from acetone treated animals have led to the pathways illustrated in Figure 2-3.

In the first step, acetone is oxidized (hydroxylation of a methyl group) to acetol by acetone monooxygenase (also called acetone hydroxylase), an activity associated the cytochrome P-450IIE1, and requires oxygen and NADPH (Casazza et al. 1984; Johansson et al. 1986; Koop and Casazza 1985; Puccini et al. 1990). Cytochrome P-45011El can be induced by fasting, experimental diabetes, or exposure to ethanol or acetone (Johansson et al. 1988; Patten et al. 1986; Puccini et al. 1990). When the rate of acetone oxidation was evaluated in microsomes with acetone added to the incubation system, microsomes from rats (Johansson et al. 1986) and mice (Puccini et al. 1990) pretreated with acetone had a 7-8 times greater rate than microsomes from control rats or mice. Thus, acetone induces its own metabolism.

The formation of acetol is common to all three pathways. Subsequent conversion of acetol to methylglyoxal in microsomes is catalyzed by acetol monooxygenase (also called acetol hydroxylase), an activity also associated with cytochrome P-450IIE1, and also requires oxygen and NADPH (Casazza et al. 1984; Johansson et al. 1986; Koop and Casazza 1985). Methylglyoxal can then be converted to D-glucose by an unidentified pathway, and/or possibly by catalysis by glyoxalase I and II and glutathione to D-lactate, which is converted to D-glucose (Casazza et al. 1984). The conversion of methylglyoxal to D-lactate by the actions of glyoxalase I and II is well established (Racker 1951), but may represent a minor pathway in the metabolism of acetone (Casazza et al. 1984; Kosugi et al. 1986; Thornalley 1990). The unidentified pathway by which methylglyoxal is converted to D-glucose

FIGURE 2-3. Metabolic Pathways for Acetone*



*From Dietz et al. 1991; adapted from Casazza et al. 1984; Kosugi et al. 1986a

may involve conversion of methylglyoxal to pyruvate by 2-oxoaldehyde dehydrogenase, an activity identified using aqueous extracts of sheep liver acetone powders (Monder 1967).

In the second and third pathways, acetol is converted to L-1,2-propanediol by an extrahepatic mechanism that has not been characterized (Casazza et al. 1984; Kosugi et al. 1986a, 1986b; Rudney 1954; Skutches et al. 1990). The two pathways then diverge from the point of production of 1,2-propanediol. In the second pathway, 1,2-propanediol formed extra-hepatically returns to the liver where it is converted to L-lactaldehyde via nicotinamide adenine dinucleotide (NADH)-dependent alcohol dehydrogenase (Casazza et al. 1984; Kosugi et al. 1986a, 1986b), and L-lactaldehyde, in turn, is converted to L-lactate (Casazza et al. 1984; Ruddick 1972; Rudney 1954) via NADH-dependent aldehyde dehydrogenase (Casazza et al. 1984). L-lactate can then be converted to D-glucose (Casazza et al. 1984). In the third pathway, the L-1,2-propanediol formed extra-hepatically returns to the liver where it is degraded by an uncharacterized mechanism to acetate and formate (Casazza et al. 1984; Ruddick 1972).

Several studies have traced the labeling patterns of ^{14}C from 2-[^{14}C]-acetone or 1,3-[^{14}C]-acetone to gluconeogenic precursors and formate to incorporation of ^{14}C into glycogen, glycogenic amino acids, fatty acids, heme, cholesterol, choline, and urea (Mourkides et al. 1959; Price and Rittenberg 1950; Sakami and LaFaye 1950). The pattern of labeling suggested the involvement of the “acetate and formate” pathway. The ultimate fate of glucose is entry into glycolysis or into the tricarboxylic acid cycle, via pyruvate and acetyl coenzyme A (CoA) with the liberation of carbon dioxide, and subsequent electron transport and oxidative phosphorylation with the production of ATP (Lehninger 1970). Fatty acids, amino acids, and glycogen may also enter stages of intermediary metabolism.

The relative importance of the three pathways in the metabolism of acetone may depend upon the amount of acetone administered. When a trace amount of 2-[^{14}C]-acetone was administered intravenously to rats, the pattern of incorporation of ^{14}C into glucose was consistent with the production of glucose via the methylglyoxal/lactate pathway (Kosugi et al. 1986a). When a higher dose of 2-[^{14}C]-acetone (325 mg/kg) was injected, the pattern of incorporation was more consistent with the 1,2-propanediol pathway. These results suggest that at low doses of acetone or endogenous acetone, the methylglyoxal and lactate pathways predominate, but at higher doses, these pathways become saturated and metabolism is shunted to the formate-acetate branch of the 1,2-propanediol pathway.

In addition to the pathways illustrated in Figure 2-3, 2,3-butanediol (Casazza et al. 1984) and isopropyl alcohol (Lewis et al. 1984) were detected in the blood of rats after oral dosing with acetone, but their position in the metabolic scheme is not clear.

That acetone is extensively metabolized has been demonstrated by the finding of high percentages of ^{14}C-carbon dioxide in the expired air of animals exposed to ^{14}C-acetone (Mourkides et al. 1959; Sakami and LaFaye 1950, 1951; Price and Rittenberg 1950; Wigaeus et al. 1982) (see Section 2.3.4).

Although the liver is the primary site of acetone metabolism, radioactive unmetabolized acetone and total radioactivity were found in blood, pancreas, spleen, thymus, heart, testis, vas deferens, lung, kidney, brain, liver, muscle, brown adipose tissue, subcutaneous adipose tissue, and intraperitoneal adipose tissue of mice after inhalation exposure to ^{14}C-acetone (Wigaeus et al. 1982) (see Section 2.3.2.1). The fraction of total radioactivity that was not unchanged acetone represented metabolites. Elimination of acetone was complete in all tissues by 24 hours after exposure, but total radioactivity, indicative of metabolites, was still present in all tissues except blood and muscle. Whether these tissues (other than the liver) were capable of metabolizing acetone or whether the metabolites themselves were distributed to the tissues was not clear. However, microsomes from the lungs of hamsters exposed to acetone in drinking water for 7 days had a 500% increased activity of aniline hydroxylase activity, an enzyme associated with cytochrome P-450IIEl (Ueng et al. 1991). Furthermore, the level of cytochrome P-450IIEl increased 6-fold in microsomes from the nasal mucosa of rabbits exposed to acetone in drinking water for 1 week (Ding and Coon 1990). In hamsters given drinking water containing acetone for 7 days (Ueng et al. 1991) or 10 days (Menicagli et al. 1990), the microsome prepared from kidneys had increased levels of cytochrome P-450 and cytochrome b,. These results suggest that acetone metabolism, which involves cytochrome P-450IIE1, may occur in the lungs and kidneys of hamsters and the nasal mucosa of rabbits. Incubation of acetone with homogenates of nasal mucosa from mice indicated that acetone was metabolized via a NADPH-dependent pathway *in vitro*, but no evidence of *in vivo* metabolism of acetone by the upper respiratory tract was found in mice, rats, guinea pigs, or hamsters (Morris 1991). Injection of pregnant rats with acetone on gestational day 19 resulted in high levels of 1,2-propanediol and acetol in the fetuses (Peinado et al. 1986). Whether these findings reflect transfer of the metabolites from the dams or metabolism of transferred or endogenous acetone by the fetuses was not resolved.

Very few differences have been found among species in the metabolism of acetone. The pathways illustrated in Figure 2-3 appear to operate in rats, mice, and rabbits. In microsomes from rabbits exposed to acetone via drinking water, it was found that the oxidation of acetol could be catalyzed by cytochromes P-4503b (IIC3), 2 (IIB6), and 4(IA2), as well as by cytochrome P-4503a (P-450IIEl) (Koop and Casazza 1985). Only cytochrome P-450IIEl could catalyze the oxidation of acetone to acetol. No studies were located regarding the ability of other isoenzymes of P-450 to catalyze these reactions in other species.

Physiological or genetic status may alter the metabolism of acetone. When nondiabetic and diabetic rats were treated by gavage with acetone at doses of 1,000, 2,000, or 4,000 mg/kg, isopropyl alcohol was detected in the blood (Lewis et al. 1984). The levels of isopropyl alcohol and acetone increased with increasing dose in the diabetic rats, although with plateaus for both acetone and isopropyl alcohol at 1,000 and 2,000 mg/kg doses, but leveled off in the nondiabetic rats, indicating either saturation of the metabolic pathway from acetone to isopropyl alcohol or a reversibility of the conversion at high doses. It was suggested that in the diabetic rats, acetone and NADH, both needed for isopropyl alcohol production from acetone, presumably via alcohol dehydrogenase, may be diverted to gluconeogenic pathways to meet the diabetic rat's need for glucose, resulting in the short plateau. The subsequent rises of both compounds at the high dose of acetone in the diabetic rats could be accounted for by greater generation of NADH from fatty acid oxidation in the diabetic rat, which reduces acetone to isopropyl alcohol, accounting for the rising level of isopropyl alcohol. Liver homogenates from mice heterozygous for the obesity gene treated with acetone were more effective in converting acetone to lactate than liver homogenates from normal homozygous mice treated with acetone (Coleman 1980). The more effective conversion by heterozygous mice may account for their prolonged survival on the starvation regimen, compared with normal mice. In pregnant and virgin rats (either fed or fasted) injected intravenously with acetone, plasma acetol levels were not significantly different between fasted and nonfasted rats, but pregnant rats had significantly lower levels than virgin rats (Peinado et al. 1986). Liver levels of acetol were also significantly lower in pregnant rats than in virgin rats. Methylglyoxal levels were very high in the livers and plasma of nonfasted rats (pregnant or virgin), but fasting resulted in much lower levels. In contrast, no major differences were found in the expiration of carbon dioxide between fasted and diabetic rats injected intraperitoneally with acetone (Mourkides et al. 1959) or in the labeling pattern of ^{14}C derived from ^{14}C-acetone into glucose among nonfasted diabetic, fasted diabetic, normal nonfasted, and normal fasted rats injected intravenously with ^{14}C-acetone (Kosugi et al. 1986a, 1986b).

2.3.4 Excretion

2.3.4.1 Inhalation Exposure

The main route of excretion of acetone is via the lungs regardless of the route of exposure. Acetone is excreted both unchanged and, following metabolism, mainly as carbon dioxide. Studies have been conducted in humans exposed by inhalation, but these studies have followed the elimination only of unchanged acetone from blood and the excretion of unchanged acetone in the expired air and urine.

In humans exposed to acetone ≤1,250 ppm for ≤7.5 hours/day in a complex protocol for 16 weeks, the concentration of acetone in venous blood was directly related to the vapor concentration and duration of exposure, and inversely related to the time elapsed following exposure (Stewart et al. 1975). The rate of elimination of acetone from blood was constant regardless of blood acetone concentration (DiVincenzo et al. 1973). Half-times for blood elimination of 3-3.9 hours have been estimated in humans exposed to 100-500 ppm for 2-4 hours (Brown et al. 1987; DiVincenzo et al. 1973; Wigaeus et al. 1981). Elimination half-times of 3.9 hours and 6.2 hours have been estimated for arterial and venous blood, respectively (Wigaeus et al. 1981). No differences in elimination half-times were found between men and women (Brown et al. 1987). The elimination from blood was found to be complete in 24 hours after a 6-hour exposure in subjects exposed to 250 ppm, in 32 hours in subjects exposed to 500 ppm, and in 48 hours in subjects exposed to 1,000 ppm (Matsushita et al. 1969b). When humans were exposed for 6 hours/day for 6 days, the blood levels of acetone rose each day and declined to background levels by the following morning each day when the exposure concentration was 250 ppm (Matsushita et al. 1969a). At an exposure concentration of 500-ppm, however, the blood levels declined each day, but not to background levels. At the end of the 6-day exposure, blood acetone levels declined to background within 2 days for the 250 ppm group and within 3 days for the 500 ppm group. From the half-time and the data on time for decline to background levels, it appears that at higher concentrations, acetone may accumulate slightly in the blood during daily intermittent exposure, as would be experienced by workers.

The rate and pattern of respiratory excretion of acetone is influenced by exposure concentration, duration, the level of physical activity during exposure, and gender. In humans exposed to acetone ≤1,250 ppm for ≤7.5 hours/day in a complex protocol for ≤6 weeks, the rate of respiratory excretion was a function of the duration, and the concentration of acetone in breath after exposure was directly

related to the time-average concentration during exposure, with constant duration (Stewart et al. 1975). The length of time after exposure in which acetone could be detected in the expired air was related to the magnitude of exposure, with acetone still readily detectable 16 hours after exposure to 1,000 or 1,250 ppm for 7.5 hours. Excretion of acetone by the lungs was complete within 20 hours postexposure in humans exposed to 237 ppm for 4 hours (Dick et al. 1989). During exposure for 2 hours, the acetone concentration in expired air rose to 20 ppm in humans exposed to 100 ppm and to 90-100 ppm in those exposed to 500 ppm (DiVincenzo et al. 1973). After exposure to 100 ppm, the expired air concentration of acetone declined biphasically over the next 7 hours to 5 ppm. However, after exposure to 500 ppm, the expired air concentration dropped sharply to 2 ppm and declined to 1 ppm over the next 7 hours. Prolonging the exposure duration to 4 hours resulted in less than a 2-fold increase in acetone levels in postexposure expired air, which may reflect a greater loss of acetone through metabolism and urinary excretion. Exercise during the exposure period increased the elimination almost 2-fold. In humans exposed to acetone at rest, during exercise at a constant workload, or during exercise with step-wise increments in workload, expiration of acetone via the lungs amounted to 70, 80, and 200 mg, respectively, at 4 hours postexposure and to 50, 80, and 200 mg, respectively, over the next 4-20 hours (Wigaeus et al. 1981). Excretion of acetone from the lungs and kidneys (combined) amounted to 16, 20, and 27% of the amount absorbed in the three respective groups of subjects. Urinary excretion amounted to only 1% of the total uptake. Women expired acetone more slowly than men after a 4-hour exposure to 127-131 ppm, but the percentages excreted by the lungs were not statistically significantly different between men and women (17.6% for men, 15.0% for women) (Nomiyama and Nomiyama 1974b).

Very little unchanged acetone is excreted in the urine (DiVincenzo et al. 1973; Kawai et al. 1992; Vangala et al. 1991; Wigaeus et al. 1981). Urinary excretion is biphasic (Pezzagno et al. 1986). Peak urinary excretion occurred between 1 and 3.5 hours after exposure (Matsushita et al. 1969b; Wigaeus et al. 1981). In male volunteers exposed to 497 or 990 ppm acetone for 4 hours, cumulative acetone excretion in urine at 18 hours after cessation of exposure was 89.5 mg, suggesting slow excretion of acetone in the urine (Vangala et al. 1991). The amount of acetone excreted in the urine is influenced by the exposure concentration, the duration of exposure, and the level of physical activity during exposure. The acetone concentration in the urine ranged from 0-17.5 mg/L at the end of the 8-hour workshift in 45 workers exposed to 0-70 ppm acetone (background urinary concentration in 343 nonexposed subjects averaged 1.5 mg/L) (Kawai et al. 1992). Acetone levels in the preshift urine samples were significantly higher than background levels when acetone exposure on the previous day

was above 15 ppm. There was no significant difference between background urine levels and preshift urine levels when the previous day's exposure was ≤15 ppm. In humans exposed for 6 hours, peak urinary levels were found within the first hour after exposure and were 5.2 mg/dL in subjects exposed to 1,000 ppm, 2.9 mg/dL in subjects exposed to 500 ppm, and 1.8 mg/dL in subjects exposed to 250 ppm (Matsushita et al. 1969b). The decline in urinary acetone to background levels occurred within 48 hours for the 1,000 ppm group, within 32 hours for the 500 ppm group, and within 24 hours in the 250 ppm group. When human subjects were exposed for 6 hours/day for 6 days, urinary levels of acetone rose each day and declined to background levels by the following morning each day when the exposure concentration was 250 ppm (Matsushita et al. 1969a). At an exposure level of 500 ppm, however, urinary levels declined each day, but not to background levels. At the end of the 6-day exposure period, urinary acetone levels declined to background within 2 days for the 250 ppm group and within 3 days for the 500 ppm group. Therefore, excretion was more complete after exposure to lower concentrations, and at higher concentrations, acetone may accumulate somewhat during daily intermittent exposure, as would be experienced occupationally. Total 24-hour urine content of acetone was 1.25 mg in subjects exposed to 100 ppm for 2 hours and 3.51 mg in subjects exposed to 500 ppm for 2 hours (DiVincenzo et al. 1973). Prolonging the duration to 4 hours in 100 ppm group resulted in a total of 1.99 mg acetone in the urine. A slight increase in the urinary content of acetone (1.39 mg) was found when humans exposed to 100 ppm for 2 hours exercised during the exposure. The nature of physical activity during exposure also influenced the urinary excretion. At 3-3.5 hours after exposure, 8.5, 8.5, and 13.4 mg were excreted by the kidney in subjects exposed at rest, during exercise at a constant workload, and during exercise with step-wise increments in workload, respectively (Wigaeus et al. 1981). Urinary excretion amounted to only 1% of the total uptake.

As in humans, acetone is excreted mainly by the lungs of animals. Studies in animals have followed the elimination of acetone from blood and tissues, excretion of acetone and carbon dioxide in expired air, and the urinary excretion of formic acid.

Blood levels of acetone were highest immediately after a 4-hour exposure of rats to acetone (Charbonneau et al. 1986b). In rats exposed to 10,000 ppm, the blood level dropped from 2,114 to 5 μg /mL in 25 hours. In rats exposed to 15,000 ppm, the blood level dropped from 3,263 μg/mL to 50 μg /rnL after 25 hours. Elimination from blood was biphasic in rats exposed to 10,000 and 15,000 ppm, perhaps indicating saturation. Elimination from blood was triphasic in rats exposed to 1,000, 2,500, or 5,000 ppm and was complete within 17-25 hours. In dogs exposed to 100, 500, or

1,000 ppm acetone for 2 hours, blood levels declined in a log-linear manner with a half-time of 3 hours, similar to that observed in humans (DiVincenzo et al. 1973). Blood levels declined from 25 mg/L immediately after exposure to 10 mg/L at 5 hours postexposure for the 1,000 ppm group, from 12 to 3 mg/L for the 500 ppm group, and from 4 to 1.5 mg/L for the 100 ppm group. Elimination of radioactivity and ^{14}C-acetone was fastest from blood, kidney, lungs, brain, and muscle tissues of mice exposed to 500 ppm ^{14}C-acetone for 6 hours, with half-times of 2-3 hours during 6 hours postexposure (Wigaeus et al. 1982) (see Section 2.3.2.1). Elimination of acetone was complete in 24 hours in all tissues, but radioactivity (indicative of metabolites) was still present in all tissues except blood and muscle. When rats were exposed for 5 days, acetone tended to accumulate in adipose tissue.

Excretion of acetone in air followed pseudo-first-order kinetics in rats exposed to <20 ppm acetone for l-7 days, while at higher concentrations, saturation kinetics were observed (Hallier et al. 1981). In rats exposed to 500 ppm ^{14}C-acetone for 6 hours, 42 μmol of radioactive acetone and 37 μmol ^{14}C-carbon dioxide were excreted in the expired air during a 12-hour postexposure period, with 95% and 85%, respectively, recovered in the first 6 hours postexposure (Wigaeus et al. 1982). Radioactive acetone accounted for 52% and radioactive carbon dioxide accounted for 48% of the expired radioactivity. The concentration of acetone in the expired breath of dogs exposed to 100, 500, or 1,000 ppm acetone for 2 hours declined in a log-linear manner (DiVincenzo et al. 1973). The breath levels were directly related to the magnitude of exposure. Breath levels declined from 1.6 ppm at 30 minutes after exposure to 0.3 ppm at 300 minutes in the l00-ppm group, from 6.8 to 1.5 ppm in the 500-ppm group, and from 15 to 4 ppm in the l,000-ppm group.

Urinary excretion of formic acid was followed for 7 days in rats exposed to 62,000 ppm acetone for 2 days. The rate of formic acid excretion was 344 μg/hour compared with 144 μg /hour in controls (Hallier et al. 1981).

2.3.4.2 Oral Exposure

In volunteers who ingested 40-60 mg/kg acetone, the elimination of acetone in expired air and urine was determined 2 hours later, and a rate of metabolism of 1.82 mg/kg/hour along with the excretion data was used to calculate that 3.54-7.38 mg/kg had been excreted and metabolized (Haggard et al.

1944). The authors estimated that 65-93% of the administered dose was eliminated via metabolism, with the remainder excreted in the urine and expired air.

The only other information regarding excretion of acetone in humans after oral exposure is from case reports of accidental or intentional ingestion of materials containing acetone plus other components that may have influenced the elimination of acetone. In a man who ingested liquid cement containing 18% acetone (231 mg/kg), 28% 2-butanone, and 29% cyclohexanone and 720 mL sake, the plasma level of acetone was ≈1,120 μg /mL 5 hours after ingestion and declined to 65 μg /mL at 18 hours, 60 μg /mL at 24 hours, and <5 μg /mL at 48 hours (Sakata et al. 1989). A first-order plasma elimination rate constant of 0.038/hour and a half-time of 18.2 hours were calculated. The urinary level of acetone decreased gradually from about 123 μg /mL at 5 hours after ingestion to about 61 yg/mL at 19 hours. In a case of a known alcoholic who had ingested nail polish remover and whose blood acetone level was 0.25 g/dL (2.5 mg/mL) upon admission to the hospital, the blood level of acetone declined in a log-linear manner to about 0.06 g/dL (0.6 mg/mL) about 86 hours after admission, with a half-life of 31 hours (Ramu et al. 1978). The calculated clearance of acetone from the lungs was 29 ml/minute or 0.39 ml/minute/kg. A half-time of 25 hours for lung clearance was calculated, which is in agreement with the observed plasma elimination half-time of 31 hours. The serum acetone level of a 30-month-old child was 445 mg/100 mL (4.45 mg/mL) 1 hour after ingestion of a 6-ounce bottle of nail polish remover (65% acetone) and declined to 2.65 mg/mL at 117 hours, to 0.42 mg/mL at 48 hours, and to 0.04 mg/mL at 72 hours (Gamis and Wasserman 1988). The halftime of acetone in this patient was 19 hours in the severe early stage and 13 hours in later stages of intoxication, which suggested to the authors greater metabolism and/or excretion in children, compared with adults.

For animals, information regarding the excretion of acetone after oral exposure is available only for rats. As is the case after inhalation exposure, acetone, mainly as carbon dioxide, is excreted primarily by the lungs. In a rat given 1.16 mg/kg ^{14}C-acetone by gavage in water, expiration of ^{14}C-carbon dioxide totaled 47.4% of the administered radioactivity over the 13.5-hour collection period (Price and Rittenberg 1950). In another experiment, a rat was given 7.11 mg/kg radioactive acetone. A small amount of radioactive acetone (10%) was found in the expired air. Radioactive carbon dioxide and acetate were also detected. In a rat made diabetic by alloxan and given 6.15 mg/kg ^{14}C-acetone, a total of 7.29% of the administered radioactivity was expired as acetone and 51.78% as carbon dioxide. Radioactive acetate was detected in the urine. These data indicate that very little acetone (<10%) was

excreted by the lungs after small doses of acetone. A major fraction was oxidized to carbon dioxide and some of the derived carbon was used for acetylation. The diabetic rat was also able to oxidize acetone, but only to ≈70% of that in the normal rat.

The dose of acetone influences the elimination of acetone from blood (Plaa et al. 1982). At a dose of 78.44 mg/kg, the maximum blood level of 200 μg /mL at 3 hours declined to 10 μg /mL at 12 hours, where it remained for the next 12 hours (data inadequate to calculate total body clearance). At a dose of 196.1 mg/kg, the maximum blood level of 400 μg /mL at 6 hours declined biphasically to 50 μg /mL at 12 hours and to 30 μg /mL at 18 hours where it remained at 24 hours (total body clearance-64 ml/hour). At a dose of 784.4 mg/kg, the maximum blood level of 900 μg /mL at 1 hour declined to 300 μg /mL at 12 hours, to 110 μg /mL at 18 hours, and to 50 μg /mL at 24 hours (total body clearance-86 ml/hour). At a dose of 1,961 mg/kg, the maximum blood level of 1,900 μg /mL at 3 hours declined slowly to 400 μg /mL at 24 hours (total body clearance-75 ml/hour). Thus total body clearance was independent of dose, but the half-time for elimination increased from 2.4 hours for 196.1 mg/kg, to 4.9 hours for 784.4 mg/kg, and 7.2 hours for 1,96 1 mg/kg.

The vehicle (corn oil or water) in which acetone is administered has little influence on the elimination of acetone from blood (Charbonneau et al. 1986a). After gavage treatment of rats with 78, 196, 392, 784, or 1,177 mg/kg acetone in corn oil or water, elimination was biphasic for the two higher doses and triphasic for the lower doses. Acetone elimination from blood declined to <5 to <l0 μg /mL by 18-26 hours for all doses, but minor differences were found between water and corn oil as vehicle. The blood concentration curves from rats given acetone in water more closely resembled those from rats exposed by inhalation.

2.3.4.3 Dermal Exposure

Information regarding excretion of acetone after dermal exposure of humans is limited, but the main route of excretion is via the lungs, with little excreted in the urine. Application of an unspecified quantity of acetone to a 12.5 cm^2 area of skin of volunteers for 2 hours/day for 4 days resulted alveolar air levels of 5-12 ppm and urinary concentrations of 8-14 μg /mL on each day (Fukabori et al. 1979). These levels declined to background levels by the next day after each exposure. Higher alveolar air and urinary levels were obtained when the daily exposure increased to 4 hour/day:

25-34 ppm in alveolar air, and 29-41 μg /mL in urine, but these levels also returned to background each day.

No studies were located regarding excretion of acetone by animals after dermal exposure.

2.3.4.4 Other Routes of Exposure

As determined in humans, physiological status may influence the disposition of endogenous and exogenous acetone. In groups of nonobese patients fasted for 3 days, obese patients fasted for 3 days, and obese patients fasted for 21 days and injected intravenously with ^{14}C-acetone, 8-29% of the urinary acetone was derived from plasma radioactive acetone (Reichard et al. 1979). The concentrations of urinary acetone were 1.2, 0.4, and 2.6 μmol/mL in 3-day-fasted nonobese, 3-day-fasted obese, and 21-day-fasted obese patients, respectively. The rates of urinary acetone excretion were 1.2, 0.4, and 1.7 μmol/minute, respectively, suggesting marked renal reabsorption or backdiffusion. The percentages of measured acetone production that could be accounted for by excretion via the lungs were 14.7, 5.3, and 25.2%, respectively. The percentages that could be accounted for by urinary excretion were 1.4, 0.6, and 1.3%, respectively. Cumulative excretion of ^{14}C-carbon dioxide during the 6-hour turnover study periods accounted for 17.4, 21.5, and 4.9%, respectively. Thus, nonobese subjects fasted for 3 days excreted more acetone at higher rates than did obese subjects fasted for 3 days. However, excretion by the obese patients fasted for 21 days exceeded that by both 3-day-fasted groups. These differences are probably related to the effect that the degree of starvation ketosis has on the metabolism and overall disposition of acetone.

In contrast, no major differences were observed among normal rats, fasted rats, and diabetic rats in the excretion of ^{14}C-carbon dioxide from the lungs after intraperitoneal injections of ^{14}C-acetone (Mourkides et al. 1959). However, the dose level influenced the pattern of metabolism and, hence, the excretion of carbon dioxide. Rats that received 9.3-22.7 mg/kg radioactive acetone rapidly metabolized acetone, as evidenced by exhalation of 24-43% of the administered radioactivity as ^{14}C-carbon dioxide within the first 3 hours after dosing. Rats that received 258-460 mg/kg radioactive acetone exhaled only 2.1-5.7% of the radiolabel as carbon dioxide in the first 3 hours, 2.8-7.8% in the next 3-6 hours, and 16-29% in the next 6-24 hours. Rats injected subcutaneously with ^{14}C-acetone also excreted the derived radioactive carbon mainly as carbon dioxide. In rats fasted for 24 hours and given 170 mg/kg radioactive acetone, 27% of the radiolabel was excreted as carbon dioxide in 4 hours

(Sakami and LaFaye 1951). Rats fasted for 48 hours before the subcutaneous dose of 174 mg/kg radioactive acetone excreted 53% of the radiolabel as carbon dioxide over a 14-hour collection period (Sakami and LaFaye 1950).

Fasted pregnant rats had an enhanced capacity for acetone elimination compared with fasted or fed virgin rats or fed pregnant rats, after intravenous dosing with 100 mg/kg (Peinado et al. 1986). While the elimination of acetone from plasma was biphasic in all groups, the fasted pregnant rats eliminated acetone at a faster rate than the other groups.

2.3.5 Mechanism of Action

As discussed in the preceding sections, acetone is readily and passively absorbed from the lungs and gastrointestinal tract and probably from the skin. Since acetone is highly water soluble, it is readily taken up by the blood and widely distributed to body tissues. Other than gastric lavage in the case of ingestion, there is no known way to interfere with the absorption of acetone. Within the liver, acetone is metabolized by three separate gluconeogenic pathways through several intermediates, but most of its intermediate or final metabolites are not considered toxic. Unmetabolized acetone does not appear to accumulate in any tissue, but is excreted mainly in the expired breath. Acetone is irritating to mucous membranes, possibly due to its lipid solvent properties, resulting in eye, nose, throat, and lung irritation upon exposure to the vapors, and skin irritation upon dermal contact (see Section 2.2). The mechanism of the narcotic effects of acetone is not known, but as a solvent, acetone may interfere with the composition of the membranes, altering their permeability to ions (Adams and Bayliss 1968). Systemically, acetone is moderately toxic to the liver and produces hematological effects. The mechanism by which acetone produces these effects is unknown. The renal toxicity may be due to the metabolite, formate, which is known to be nephrotoxic (NTP 1991) and is excreted by the kidneys (Hallier et al. 1981). Furthermore, the renal toxicity, which appears to be specific for male rats, may involve $\alpha_{2\mu}$-globulin syndrome, as hyaline droplet formation was associated with the nephropathy observed in male rats in the American Biogenics Corp. (1986) study. Acetone also causes increases in liver and kidney weight, probably through the induction of microsomal enzymes, which would increase the weight of the organs by virtue of the increased protein content. Acetone also causes reproductive effects in male rats and is fetotoxic. Although the exact mechanism for many of the effects of acetone is not known, distribution studies in mice indicate that acetone and metabolites are found in all of the

target organs (Wigaeus et al. 1982). Acetone and some of its metabolites were also transferred to rat fetuses after the dams were exposed to acetone (Peinado et al. 1986).

One of the major effects of acetone is the potentiation of the toxicity of other chemicals (see Section 2.6). Pretreatment with acetone has been shown to potentiate the hepatotoxicity and nephrotoxicity of carbon tetrachloride and chloroform (Brown and Hewitt 1984; Charbonneau et al. 1985; 1986a, 1986b, 1988, 1991; Folland et al. 1976; Hewitt et al. 1980; Hewitt et al. 1987; Plaa et al. 1973, 1982; Plaa and Traiger 1972; Sipes et al. 1973; Traiger and Plaa 1972, 1974) by inducing particular forms of cytochrome P-450, especially cytochrome P-45OIIE1, and associated enzyme activities (Brady et al. 1989; Johansson et al. 1988; Kobusch et al. 1989). The induction of these enzymes leads to the enhanced metabolism of carbon tetrachloride and chloroform to reactive intermediates capable of causing liver and kidney injury. Acetone enhances the formation of carboxyhemoglobin by dichloromethane via induction of cytochrome P-450IIE1, leading to enhanced metabolism of dichloromethane to carbon monoxide (Pankow and Hoffmann 1989). Acetone also potentiates the hepatotoxicity of acetaminophen (Jeffery et al. 1991; Moldeus and Gergely 1980; Liu et al. 1991), N-nitrosodimethylamine and N-nitrosodiethylamine (Hong and Yang 1985; Lorr et al. 1984; Sipes et al. 1978;), thiobenzamide (Chieli et al. 1990), oxygen (Tindberg and Ingelman-Sundberg 1989), and chromate (Cr[VI]) (Mikalsen et al. 1991); the genotoxicity of N-nitrosodimethylamine (Glatt et al. 1981; Yoo and Yang 1985; Yoo et al. 1990); the hematotoxicity of benzene (Johansson et al. 1988; Johansson and Ingelman-Sundberg 1988; Schnier et al. 1989); and the lethality of acetonitrile (Freeman and Hayes 1985; 1988) by inducing cytochrome P-450IIEl. The hepatotoxic and nephrotoxic effects of dibromochloromethane and bromodichloromethane (Hewitt et al. 1983) and the hepatotoxic effects of 1,l ,Ztrichloroethane (MacDonald et al. 1982a, 1982b), 1, 1-dichloroethene (Hewitt and Plaa 1983; Jaeger et al. 1975), and dichlorobenzene (Brondeau et al. 1989) are also enhanced by acetone. The exact mechanisms for these interactions are not clear, but the involvement of mixed function oxidases has been implicated. The renal toxicity of N-(3,5-dichlorophenyl) succinimide (a fungicide) is potentiated by acetone by the induction of cytochrome P-450IIEl (Lo et al. 1987).

In other interactions, acetone enhances the neurotoxicity of ethanol by a proposed mechanism whereby acetone inhibits the activity of alcohol dehydrogenase, a reaction responsible for 90% of the elimination of ethanol (Cunningham et al. 1989). Acetone also potentiates the neurotoxicity and reproductive toxicity of 2,5-hexanedione (Ladefoged et al. 1989; Lam et al. 1991; Larsen et al. 1991).

The exact mechanism for these interactions is not clear but appears to involve decreased body clearance of 2,5-hexanedione by acetone (Ladefoged and Perbellini 1986).

These interactions and mechanisms are discussed more fully in Section 2.6.

2.4 RELEVANCE TO PUBLIC HEALTH

Acetone is a highly volatile, highly water-soluble aliphatic ketone. Acetone is readily absorbed by the lungs and gastrointestinal tract, taken up the blood, and widely distributed to organs and tissues of the body. Acetone can also be absorbed dermally. Acetone is metabolized mainly in the liver by three separate gluconeogenic pathways, leading to the production of glucose with subsequent liberation of carbon dioxide. With the possible exception of formate, none of the intermediate metabolites appear to be toxic. Acetone and acetone-derived carbon dioxide are excreted mainly in the expired breath, with very little acetone excreted in the urine. Elimination of acetone is generally complete within l-3 days, depending on the dose and duration of exposure, and has little tendency to accumulate. Acetone is also produced endogenously in the body during lipid metabolism, which increases with fasting. Normal levels of acetone in breath, blood, and urine can vary widely depending on a number of factors, such as infancy, pregnancy, lactation, diabetes, physical exercise, dieting, physical trauma, and alcohol consumption.

As a solvent, acetone is irritating to mucous membranes, and exposure to the vapors can irritate the respiratory system and eyes. Acetone has anesthetic properties and causes headaches, lightheadedness, confusion, dizziness, and can lead to unconsciousness and coma in humans at high enough exposure levels. Neurobehavioral effects have been observed in humans exposed acutely by inhalation either in the workplace or in laboratory experiments. Hematological effects, which might indicate immunological effects, have been observed in humans exposed acutely to acetone in laboratory experiments. Acute inhalation of acetone may shorten the menstrual cycle. Exposure to acetone vapor can also lead to increased pulse rates, gastrointestinal irritation, nausea, vomiting, and hemorrhage. However, the odor threshold of acetone (l00-140 ppm) and the feelings of irritancy are excellent warning properties that generally preclude serious inhalation over-exposure. Accidental or intentional ingestion of acetone can cause erosions in the mouth, coma, and diabetes-like symptoms. Acute dermal exposure of humans to liquid acetone resulted in degenerative changes in the epidermis, and a case of contact dermatitis was reported.

Acetone is also irritating to the respiratory system of animals and produces narcosis, coma, and behavioral effects upon acute inhalation exposure. Hematological effects have also been observed in animals. Acute inhalation exposure of animals during gestation has resulted in decreased fetal body weight and increased incidences of late resorption and reduced ossification. Acute oral exposure of animals resulted in enzyme induction in the respiratory system, bone marrow, gastrointestinal tract, kidney, and liver, increased liver weight and bone marrow hypoplasia, degeneration of apical microvilli in renal tubules, and reduced insulin-stimulated glucose oxidation in adipose tissue. Acute oral exposure of animals also resulted in neurological effects, such as languid behavior, prostration before death, reproductive effects (reduced reproduction index and increased duration of gestation), and developmental effects (reduced postnatal pup survival and reduced fetal weight). Intermediate-duration oral studies in animals have found liver effects, hematological effects, nephropathy, and effects on male reproductive organs. Ocular exposure to undiluted or concentrated acetone has produced severe cornea1 burns and necrosis, and dermal exposure of animals to acetone has resulted in reports of cataracts in one species and only a single report of amyloidosis. These observations have not been made by other investigators or in other species, despite many experiments involving dermal exposure of test animals to acetone.

The relevance of the liver, reproductive, and developmental effects to humans is not known, since these end points have not been sufficiently examined in humans. However, few species differences exist in the toxicokinetics of acetone, suggesting that these effects might be of concern for humans. The renal effects may be specific for male rats, and the cataract formation may be specific for guinea pigs, The relevance of amyloidosis in completely unknown. Acetone appears to have no delayed toxic effects. Acetone may be weakly genotoxic, but the majority of genotoxicity assays were negative. Acetone has not been studied for carcinogenicity by the inhalation or oral route, but it was not tumorigenic when tested alone, when tested as a tumor initiator, or when tested as a tumor promoter in skin painting studies.

One of the most studied effects of acetone is the induction of microsomal enzymes, particularly of cytochrome P-450IIEl. Acetone induces its own metabolism by this induction, and potentiates the toxicity of numerous other chemicals by enhancing the metabolism, which depends on cytochrome P-450IIE1, to reactive intermediates. The induction of cytochrome P-45011El by acetone has been documented in many species, and therefore poses a concern for humans exposed to acetone and the chemicals whose toxicity is potentiated by acetone.

Based on results of animal studies, which indicate that male animals are more susceptible than female animals, men may be more susceptible than women to the hematological, hepatic, and renal effects, and effects on reproductive organs. Furthermore, acetone may exacerbate preexisting hematological, liver, kidney, or reproductive disorders in humans. The very young and the elderly may be more susceptible. Fasting and diabetes increases endogenous levels of acetone in humans, suggesting that dieters and diabetics may have a higher body burden, and additional exposure to acetone may make them more susceptible to any effects. Results in animals suggest that the rate of acetone metabolism is slower in pregnancy, making pregnant animals more susceptible, and this susceptibility might apply to humans.

The general population can be exposed to acetone in the air, in contaminated water and soil, and by ingestion of food containing acetone. As discussed in chapter 5, acetone is emitted into the air from plants and trees, volcanic eruptions, forest fires, automobile exhaust, chemical manufacturing, tobacco smoke, wood burning and pulp, refuse in polyethylene combustion, petroleum storage facilities, landfill sites, and solvent use. Acetone is also found in nail polish remover, paint remover, glue, and cleaning agents. Acetone is released into water by chemical manufacturing industries and energy-related industries. Acetone can be released to soil from municipal and industrial landfills, from atmospheric deposition, from disposed agricultural, food and animals wastes, and from household septic tanks. The half-life of acetone in air due to reactions with hydroxyl radical and photolysis is 22 days. The relatively long half-life permits transport to areas remote from the source. Because of its high water solubility, precipitation can remove acetone from the air to surface water and soil. In water and soil acetone undergoes microbial degradation but can also evaporate back into the atmosphere, depending on the moisture content of soil. Adsorption to soil and sediment is inconsequential. Acetone does not accumulate in fish or other aquatic or terrestrial organisms. Ambient air levels of acetone average $\leq$l ppb (v/v) in remote areas, 3 ppb in rural areas, 6.9 ppb in urban air, and 8 ppb in indoor air due to the use of household consumer products. Acetone levels can be $\leq$30 ppb in seawater, $\leq$40 ppb in river water, $\leq$62 ppm in industrial landfill leachate, 0.3-3,000 ppb in well water, and $\leq$1 ppb in finished drinking water. Occupations in which workers may be exposed to higher levels of acetone include paint manufacturing, plastics manufacturing, artificial fiber industries, shoe factories, professional painting, and commercial cleaning. The OSHA standard for a time-weighted average exposure to acetone over an 8-hour workday and 40-hour workweek is 750 ppm, and the short-term exposure limit is 1,000 ppm.

Minimal Risk Levels for Acetone

Inhalation MRLs

- An MRL of 26 ppm has been derived for acute-duration inhalation exposure (14 days or less) to acetone.

The MRL was based on a LOAEL of 237 ppm for 4 hours for neurobehavioral effects in an experimental study in humans (Dick et al. 1989). In this study, groups of 11 men and 11 women were exposed for 4 hours to acetone at a concentration of 237 ppm. Statistically significant changes in performance from controls were seen in two measures of auditory tone discrimination (increased response time and increased false alarms) and in anger and hostility. Neurological and behavioral effects have also been observed in humans exposed to 250 ppm acetone for 6 hours or repeatedly for 6 hours/day for 6 days (Matsushita et al. 1969a, 1969b). Effects included lack of energy, general weakness, delayed visual reaction time, and headache. Although irritation of the nose, throat, and trachea was reported in humans exposed to 100 ppm for 6 hours (Matsushita et al. 1969b), other studies in humans report respiratory irritation only at higher levels (≥250 ppm) for longer durations (Matsushita et al. 1969a; Nelson et al. 1943; Raleigh and McGee 1972; Ross 1983). Furthermore, the reporting of these irritating effects was subjective, and only six volunteers were exposed to 100 ppm.

- An MRL of 13 ppm has been derived for intermediate-duration inhalation exposure (15-364 days) to acetone.

The MRL was based on a LOAEL of 1,250 ppm for neurological effects in volunteers in a 6-week study (Stewart et al. 1975). There was a statistically significant increase in amplitude of the visual evoked response in subjects tested following exposure to 1,250 ppm. Neurological and behavioral effects (lack of energy, general weakness, delayed visual reaction time, and headache) have also been observed in humans exposed to 250 ppm acetone for 5.25 hours or repeatedly for 6 hours/day for 6 days (Matsushita et al. 1969a, 1969b). Increased anger and hostility, as well as increased response time and rate of false negatives in a test of auditory tone discrimination, were reported in volunteers exposed to 237 ppm for 4 hours (Dick et al. 1989). No other effects were detected in humans or animals exposed to acetone for intermediate durations (Bruckner and Peterson 1981b; Stewart et al. 1975)

- An MRL of 13 ppm has been derived for chronic-duration inhalation exposure (365 days or more) to acetone.

The MRL was based on a LOAEL of 1,250 ppm for neurological effects in volunteers in a 6-week study (Stewart et al. 1975). There was a statistically significant increase in amplitude of the visual evoked response in subjects tested following exposure to 1,250 ppm. Neurological and behavioral effects (lack of energy, general weakness, delayed visual reaction time, and headache) have also been observed in humans exposed to 250 ppm acetone for 5.25 hours or repeatedly for 6 hours/day for 6 days (Matsushita et al. 1969a, 1969b). Increased anger and hostility, as well as increased response time and rate of false negatives in a test of auditory tone discrimination, were reported in volunteers exposed to 237 ppm for 4 hours (Dick et al. 1989). A health survey of workers exposed to acetone between 3 months and 23 years revealed no systemic effects (Ott et al. 1983a, 1983c).

Oral MRLs

- An MRL of 2 mg/kg/day has been derived for intermediate-duration oral exposure (15-364 days) to acetone.

The MRL was based on a NOAEL of 200 mg/kg/day for macrocytic anemia in rats treated with acetone in the drinking water for 13 weeks (Dietz et al. 1991; NTP 1991). The LOAEL was 400 mg/kg/day. Hematological effects have been observed in humans exposed by inhalation to acetone (Matsushita et al. 1969a, 1969b), in rats exposed to 6,942 mg/kg/day in the drinking water for 14 days (Dietz et al. 1991; NTP 1991), and in rats treated by gavage with 2,500 mg/kg/day for 93-95 days (American Biogenics Corp. 1986). Male rats treated with 900 mg/kg/day in the drinking water also had increased incidence of age-related nephropathy, which increased in severity as the dose increased (Dietz et al. 1991; NTP 1991). These renal lesions were not seen in female rats or in male or female mice similarly treated. In male rats treated by gavage with $\geq$500 mg/kg/day, but not 100 mg/kg/day, an enhancement of age-related nephropathy, accompanied by hyaline droplet accumulation, was observed (American Biogenics Corp. 1986). Female rats had enhanced age-related nephropathy only at the highest dose (2,500 mg/kg/day), but no evidence of hyaline droplet accumulation was found. Because the mechanism for acetone-induced renal toxicity has not been

elucidated, but may be specific for male rats, renal end points have not been considered for the derivation of MRLs for acetone.

An MRL has not been derived for acute-duration oral exposure (14 days or less) to acetone because a suitable NOAEL or LOAEL for a sensitive end point has not been sufficiently characterized, Such effects as bone marrow hypoplasia, reduced insulin-stimulated glucose oxidation, and hepatocellular hypertrophy were observed in rats or mice exposed to acetone in the drinking water for acute durations (Dietz et al. 1991; NTP 1991; Skutches et al. 1990), but the NOAEL and LOAEL values for these effects are close to or fall within the range of LD_{50} values for rats administered acetone by gavage (Freeman and Hayes 1985; Kimura et al. 1971). Degeneration of apical microvilli in renal tubules was observed in male rats treated once by gavage with 871 mg/kg (Brown and Hewitt 1984), but incidences were not reported. Furthermore, as discussed above, acetone-induced renal lesions may be specific for male rats and may not represent a suitable end point to consider for MRL derivation for acetone.

An MRL was not derived for chronic-duration oral exposure (365 days or more) to acetone because no chronic-duration studies were located.

Death. No studies were located regarding death of humans after dermal exposure to acetone. A retrospective mortality study of workers exposed to time-weighted-average acetone concentrations of 380-1,070 ppm at a cellulose fiber plant where acetone was used as the only solvent found no significant excess risk of death from any cause compared with rates for the U.S. general population (Ott et al. 1983a, 1983b). Furthermore, in the 1991 Annual Report of the American Association of Poison Control Centers National Data Collection System, no fatalities were reported among 1,124 cases of accidental or intentional ingestion of acetone (Litovitz et al. 1992). However, inhalation of saturated atmospheres of acetone or accidental or intentional ingestion of acetone can lead to unconsciousness if doses are sufficiently high, and death could ensue if the subjects do not receive medical intervention. Two workers became unconscious after a single accidental exposure to acetone at high concentrations (>1,200 ppm) (Ross 1973). Coma developed in patients who had hip casts applied with acetone present in the setting fluid (Chatterton and Elliott 1946; Fitzpatrick and Claire 1947; Harris and Jackson 1952; Renshaw and Mitchell 1956; Strong 1944). These patients were exposed to acetone by inhalation during the application and from evaporation of acetone from the casts after application, but dermal exposure also could have occurred. In most cases of accidental or

intentional ingestion, the quantity of acetone consumed is not known. In one case, a man became comatose after ingesting 100 mL of liquid cement containing 15% polyvinyl chloride, 18% acetone, 28% 2-butanone, and 39% cyclohexanone, along with 750 mL sake (Sakata et al. 1989). The dose of acetone ingested would be approximately 231 mg/kg, but the other components in the cement and the sake could have contributed to his condition. In another case, a man became comatose after ingesting 200 mL of pure acetone (2,241 mg/kg) (Gitelson et al. 1966). Since the patients received medical treatment, none died.

High concentrations of acetone in air are required to produce death in animals. An 8-hour LC_{50} value of 21,091 ppm and a 4-hour LC_{50} value of 31,944 ppm were found for female rats (Pozzani et al. 1959). Death of animals has occurred after acute inhalation exposure to l0,000-50,000 ppm (Bruckner and Peterson 1981b; Smyth et al. 1962; Specht et al. 1939). No information was located regarding levels of acetone that would result in death of animals after inhalation exposure for longer durations. Acute oral LD_{50} values in rats have ranged from 1,726 to 9,833 mg/kg, depending on age and strain (Freeman and Hayes 1985; Kimura et al. 1971; Pozzani et al. 1959; Smyth et al, 1962). In general, newborn rats are the most sensitive, followed by 14-day-old rats, older adult rats, and younger adult rats (Kimura et al. 1971), and Sprague-Dawley rats (Freeman and Hayes 1985; Kimura et al. 1971) appeared to be more sensitive than Wistar rats (Smyth et al. 1962) or Nelson rats (Pozzani et al. 1959). Acute oral LD_{50} values of 5,250 mg/kg for male mice (Tanii et al. 1986) and 3,687 mg/kg for guinea pigs (Striegel and Carpenter 1961) have been reported. Doses of 3,922-7,844 mg/kg (Walton et al. 1928) and 7,500-8,000 mg/kg (Albertoni 1884) have been reported to be fatal to rabbits and puppies, respectively. An acute oral dose of 2,400 mg/kg was fatal to one of four pregnant mice, and more pregnant mice died at higher doses (EHRT 1987), but no effect on mortality was found in nonpregnant mice exposed to acetone in drinking water at doses $\leq$ 1,298 mg/kg/day for 13 weeks (NTP 1991). Therefore, pregnant mice may be more susceptible than nonpregnant mice. Likewise, in intermediate-duration studies, no effects on mortality were found in rats exposed to acetone by gavage (American Biogenics Corp. 1986) or in drinking water (NTP 1991) at doses $\leq$3,400 mg/kg/day. In studies attempting to determine dermal LD_{50} values for animals, the highest doses used did not result in death. Therefore, the LD_{50} values for dermal exposure were >9.4 mL/kg for guinea pigs (Roudabush et al. 1965) and >20 mL/kg for rabbits (Smyth et al. 1962).

The lethality of acetone is related to its narcotic properties, since prostration and unconsciousness usually precede death (Albertoni 1984; EHRT 1987; Freeman and Hayes 1985; Specht et al. 1939;

Walton et al. 1928). It is unlikely that humans would die after exposure to acetone at ambient air, soil, or water levels, or at levels present at hazardous waste sites. Accidental occupational exposure and accidental or intentional ingestion of acetone could conceivably lead to death if the victims do not receive medical attention. In the case of the worker who became unconscious at an acetone level of 900 ppm, the OSHA standard of 750 ppm (OSHA 1989) was exceeded.

Systemic Effects

Respiratory Effects. Acetone does not appear to produce respiratory effects in humans or animals after oral or dermal exposure. As is common with solvent exposure, the respiratory effects of acetone observed in humans exposed by inhalation are related to the irritating properties of acetone. Experimental subjects experienced irritation of the nose, throat, and trachea at acute inhalation concentrations ≥100 ppm for 6 hours (Matsushita et al. 1969b), but this appears to be related to the duration of exposure, as people exposed to 200 ppm for 3-5 minutes experienced no irritation and estimated that they could tolerate this concentration for 8 hours (Nelson et al. 1943). Workers exposed to higher concentrations also complained of nose, throat, and lung irritation (Raleigh and McGee 1972; Ross 1973). Individual sensitivity to acetone-induced respiratory irritation appears to be highly variable (Nelson et al. 1943). Pulmonary function testing of volunteers exposed to ≤1,250 ppm acetone intermittently for various durations in a complex protocol revealed no abnormalities caused by the exposure (Stewart et al. 1975).

Acetone is also irritating to the respiratory system of animals after acute inhalation exposure. Guinea pigs that died after exposure to high concentrations of acetone had pulmonary congestion and edema due to the irritating properties of acetone, and hemorrhage of the lung, probably as a consequence of death (Specht et al. 1939). An RC_{50} of 77,516 ppm was found for mice (Kane et al. 1980). The decrease in respiratory rate is due to sensory irritation and is typical of sensory irritants, No intermediate-duration inhalation studies examined animals for respiratory effects, and no chronic-duration inhalation studies in animals were located. Changes in respiratory rates or irregular respiration were observed in rabbits dosed orally with ≥3,922 mg/kg (Walton et al. 1928) and dogs dosed orally with 4,000 mg/kg (Albertoni 1884), but were accompanied by signs of narcosis. Acute oral exposure of rabbits to 863 mg/kg/day resulted in the induction of microsomal enzymes in the nasal mucosa (Ding and Coon 1990), but induction of microsomal enzymes is a normal physiological response to xenobiotics and is not adverse. However, acetone potentiates the toxicity of chemicals by

enzyme induction (see Section 2.6). While it is conceivable that acetone might potentiate the toxicity of chemicals toxic to the respiratory system, no such interactions were located.

It is unlikely that people would experience respiratory irritation after exposure to acetone at ambient air, soil, or water levels, or at levels present at hazardous waste sites. Occupational exposure to acetone can cause respiratory irritation, but other respiratory effects are unlikely. The OSHA time-weighted average standard to protect against irritation is 750 ppm, and the short-term exposure limit is 1,000 ppm (OSHA 1989), which is higher than the exposure levels producing irritation in laboratory subjects.

Cardiovascular Effects. No studies of workers located reported cardiovascular effects of acetone unrelated to narcosis. A retrospective mortality study of workers exposed to time-weighted-average acetone concentrations of 380-1,070 ppm at a cellulose fiber plant where acetone was used as the only solvent found no significant excess risk of death from circulatory system disease or ischemic heart disease compared with rates for the U.S. general population (Ott et al. 1983a, 1983b). Electrocardiography of volunteers exposed to ≤1,250 ppm acetone intermittently for various durations in a complex protocol revealed no abnormalities caused by the exposure (Stewart et al. 1975). Patients exposed to acetone by inhalation and/or dermally after application of casts for which acetone was used in the setting solution commonly had high pulse rates (120-l60/minute) (Chatterton and Elliott 1946; Hift and Pate1 1961; Pomerantz 1950; Renshaw and Mitchell 1956). No gross heart lesions were found in guinea pigs exposed acutely by inhalation to lethal concentrations (Specht et al. 1939), and no histopathological heart lesions were found in rats exposed by inhalation to acetone for 2-8 weeks (Bruckner and Peterson I981b) or in rats or mice exposed orally in intermediate-duration studies (American Biogenics Corp. 1986; NTP 1991). However, amyloidosis was observed in the hearts of mice whose lumbo-sacral regions were painted twice weekly with an unspecified quantity of acetone for 12 months (Barr-Nea and Wolman 1977). The significance of the amyloidogenic effect of acetone to humans is unknown since no clinical data regarding amyloidosis in humans who come in frequent dermal contact with acetone, such as manicurists, are available.

The effects of acetone on the perfused isolated hearts of rabbits (Raje 1980) and the isolated right atrium of rats (Chentanez et al. 1987) have been investigated *in vitro.* Acetone at concentrations of 0.5 and 1.0% (v/v) in the perfusate inhibited the force of cardiac contractions and increased the heart rate of rabbits in a dose-related manner (Raje 1980). Acetone also increased the coronary flow, but

not in a dose-related manner. The author stated, however, that the concentrations of acetone were physiologically unrealistic. Acetone at concentrations of l0-210 mM in the bathing solution increased the atria1 contraction rate of the isolated rat atrium in a dose-dependent manner (Chentanez et al. 1987). At concentrations >210 mM, the atria1 contraction rate gradually decreased to the normal control value. At concentrations of l0-750 mM, acetone resulted in a dose-dependent increase in the release of norepinephrine from the right atrium. The dose-response curves for atria1 contraction and peak norepinephrine release were parallel at acetone concentrations of l0-210 mM, but norepinephrine release still increased at concentrations ≤500 mM, while the atria1 contraction rate decreased at ≥210 mM. The results indicated that the increase in atria1 contraction rate may be due in part to an increase in norepinephrine release from the atria1 sympathetic nerve terminals at the lower concentrations, but the higher concentrations may have been too toxic for norepinephrine to exert a further effect on atria1 contraction. These *in vitro* studies were conducted to investigate the mechanism of tachycardia in glue-sniffers to determine whether acetone, one of the components of glue, was the component responsible for the tachycardia. Other components of glue, however, could also cause tachycardia. These *in vitro* experiments suggest that acetone may cause tachycardia *in vivo*, but tachycardia was not demonstrated by electrocardiography in the subjects exposed to ≤1,250 ppm in the experiments by Stewart et al. (1975), and rapid heart rates were not reported as one of the subjective symptoms in workers or experimental subjects.

The weight of evidence for cardiovascular effects in humans and animals exposed to acetone *in vivo* suggest that it is unlikely that people would experience cardiovascular effects after exposure to acetone at ambient air, soil, or water levels, or at levels present at hazardous waste sites.

Gastrointestinal Effects. Nausea and vomiting of blood have been observed in patients who had hip casts applied with acetone present in the setting fluid (Chatterton and Elliott 1946; Fitzpatrick and Claire 1947; Harris and Jackson 1952; Hift and Pate1 1961; Pomerantz 1950; Renshaw and Mitchell 1956; Strong 1944). The vomitus contained blood several hours after the onset of vomiting; therefore, the gastrointestinal hemorrhage may have been due to trauma of repeated vomiting. These patients were exposed to acetone by inhalation during the application and from evaporation of acetone from the casts after application, but dermal exposure also could have occurred. None of the workers examined in on-site medical evaluations (Raleigh and McGee 1972) or experimental subjects exposed to ≤1,250 ppm complained of nausea or vomiting (DiVincenzo et al. 1973; Matsushita et al. 1969a, 1969b; Stewart et al. 1975). However, complaints of nausea and intermittent vomiting were reported

by workers with accidental exposure to acetone (>12,000 ppm) (Ross 1973). It is possible that nausea and vomiting are secondary to central nervous system toxicity. Effects on the stomach or intestines were not reported in case reports of people who ingested acetone intentionally or accidentally (Gamis and Wasserman 1988; Gitelson et al. 1966; Sakata et al. 1989), but a man who intentionally drank ≈200 mL of pure acetone (≈2,241 mg/kg) had a red and swollen throat and erosions in the soft palate and entrance to the esophagus (Gitelson et al. 1966).

No gross stomach lesions were found in guinea pigs exposed acutely by inhalation to lethal concentrations (Specht et al. 1939), and no histopathological stomach or intestinal lesions were found in rats or mice exposed orally in intermediate-duration studies (American Biogenics Corp. 1986; NTP 1991). Although cytochrome P-450 enzyme levels were significantly elevated in duodenal and jejunal microsomes from rats exposed to acetone in drinking water for 3 days (Carriere et al. 1992), induction of microsomal enzymes is not considered an adverse effect.

People exposed to acetone at work could experience nausea and vomiting, but levels of acetone in ambient air, soil, or water at hazardous waste sites are not likely to be high enough to cause nausea and vomiting.

Hematological Effects. Acetone exposure has caused hematological effects in both humans and animals. In men exposed experimentally to ≥500 ppm acetone for 6 hours or for 6 hours/day for 6 days, significantly increased white blood cell counts, increased eosinophil counts, and decreased phagocytic activity of neutrophils were observed, compared with controls (Matsushita et al. 1969a, 1969b). No women were included in these studies. However, experimental studies that examined hematological indices did not find any abnormalities in men (women not included in study) at exposure levels of 500 ppm for 2 hours (DiVincenzo et al. 1973) or in men or women at exposure levels ≤1,250 ppm acetone for ≤7.5 hours for various durations (Stewart et al. 1975). The reasons for these different results are not known. In a health evaluation survey of 245 workers exposed to time-weighted- average acetone concentrations of 380-1,070 ppm at a cellulose fiber production plant where acetone was used as the only solvent, all hematological parameters were within normal limits (Ott et al. 1983a, 1983c).

Oral exposure of rats to acetone was found to significantly increase the level of cytochrome P-45IIE1 in bone marrow microsomes (Schnier et al. 1989). While microsomal enzyme induction is not

generally considered an adverse effect, the hemotoxicity of benzene depends upon its activation by cytochrome P-450IIEl (see Section 2.6); therefore, acetone may potentiate the hemotoxicity of benzene by this mechanism.

Hematological effects observed in animals consisted of bone marrow hypoplasia in male rats, but not female rats, exposed to acetone in the drinking water for 14 days at 6,942 mg/kg/day (NTP 1991); evidence of macrocytic anemia in male rats at ≥400 mg/kg/day and nonspecific hematological effects not indicative of anemia in female rats at 3,100 mg/kg/day via drinking water for 13 weeks (NTP 1991); increased hemoglobin, hematocrit, and mean cell volume in male rats, but not female rats, treated by gavage at 2,500 mg/kg/day for 46 days, and in both males and females at this dose for 13 weeks (American Biogenics Corp. 1986). In mice treated at ≤12,725 mg/kg/day for 14 days, it was not clear whether bone marrow was examined, but in the 13-week study, no hematological effects or histologically observable lesions in hematopoietic tissues were found in mice (NTP 1991). Thus, sex differences exist in the hematological effects of acetone in animals, and species differences exist in the hematological effects of acetone among animals and between animals and humans.

The weight of evidence indicates that acetone exposure might produce hematological effects in humans. Based on animals studies, men might be more susceptible than women. Whether levels of acetone in ambient air, soil, or water, or levels present at hazardous waste sites are high enough to cause hematological effects is not known, but occupational exposure levels may be high enough, as the OSHA time-weighted average standard is 750 ppm and the short-term exposure limit is 1,000 ppm (OSHA 1989).

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans after inhalation, oral, or dermal exposure in humans or animals after inhalation or dermal exposure to acetone. Histological examination of rats and mice in intermediate-duration drinking water or gavage studies did not find any evidence of musculoskeletal effects (American Biogenics Corp. 1986; NTP 1991)

In appears unlikely that exposure of humans to acetone in any setting would result in musculoskeletal effects.

Hepatic Effects. No indication that acetone caused hepatic effects in humans was found in controlled studies of volunteers based on analysis of clinical chemistry parameters (DiVincenzo et al. 1973; Stewart et al. 1975). Furthermore, none of the located studies of workers or case reports documented hepatic effects. In a health evaluation survey of 245 workers exposed to time-weighted-average acetone concentrations of 380-1,070 ppm at a cellulose fiber production plant where acetone was used as the only solvent, all clinical blood chemistry parameters (aspartate aminotransferase, alanine aminotransferase, lactic dehydrogenase, alkaline phosphatase, total bilirubin, and albumin) were within normal limits (Ott et al. 1983a, 1983c).

Inhalation and oral studies in animals have found only mild to moderate hepatic effects. Guinea pigs acutely exposed by inhalation to lethal concentrations had fatty deposits in the liver, which were considered toxicologically insignificant (Specht et al. 1939). Mice exposed intermittently to 6,600 ppm acetone during gestation had significantly increased absolute and relative liver weights, indicative of maternal toxicity (NTP 1988). The increased liver weight could have been associated with enzyme induction. No increases in serum levels of hepatic enzymes indicative of liver injury were found in acute oral studies in rats (Brown and Hewitt 1984; Charhonneau et al. 1986b; Plaa *et* al. 1982), and no histopathological liver lesions were found in an acute oral study in mice (Jeffery et al. 1991). Liver effects observed in animals orally exposed to acetone consisted of dose-related increased liver weight in mice exposed to acetone in drinking water at ≥965 mg/kg/day and hepatocellular hypertrophy at ≥3,896 mg/kg/day for 14 days, probably associated with microsomal enzyme induction (NTP 1991); significantly increased levels of serum alanine aminotransferase in male rats, but not female rats, at a gavage dose of 2,500 mg/kg/day for 46 days or 13 weeks, and significantly increased liver weights in female rats at ≥500 mg/kg/day and in male rats at 2,500 mg/kg/day for 13 weeks (American Biogenics Corp. 1986); and significantly increased liver weights in both sexes of rats at the same drinking water concentration (1,600 mg/kg/day for females, 1,700 mg/kg/day for males) and in female mice, but not male mice, at 11,298 mg/kg/day for 13 weeks (NTP 1991). Histological examination of the liver of rats and mice in the 13-week studies revealed no evidence of liver pathology (American Biogenics Corp. 1986; NTP 1991). The increased liver weight in mice in the 13-week study was not associated with hepatocellular hypertrophy as was seen in the 14-day study, suggesting a development of tolerance (NTP 1991). In the absence of histologically observable liver lesions in rats in the 13-week gavage study (American Biogenics Corp. 1986), the toxicological significance of the increased serum level of alanine aminotransferase in the same study is questionable. Amyloidosis was observed in the livers of mice whose lumbo-sacral regions were painted twice

weekly with an unspecified quantity of acetone for 12 months (Barr-Nea and Wolman 1977). The significance of the amyloidogenic effect of acetone to humans is unknown, since no clinical data regarding amyloidosis in humans who come in frequent dermal contact with acetone, such as manicurists, are available.

As mentioned above, increased liver weight was probably associated with hepatic microsomal enzyme induction. Numerous acute oral and inhalation studies have demonstrated that acetone induces hepatic microsomal monooxygenase activities, particularly activities associated with cytochrome P-450IIEl (see Sections 2.2.1.2 and 2.2.2.2). Acetone potentiates the hepatotoxicity of carbon tetrachloride and chloroform and other chemicals (see Section 2.6) by inducing microsomal enzymes that metabolize these chemicals to reactive intermediates (see Sections 2.3.5 and 2.6).

Therefore, it is likely that people would develop no or only minimal liver effects by exposure to acetone alone at ambient air, soil, or water levels, at levels present at hazardous waste sites, or in occupational settings. However, exposure to acetone alone is extremely unlikely in these scenarios, since many other chemicals are also present in air, soil, and water and at hazardous waste sites, and workers are commonly exposed to other solvents and chemicals that produce hepatotoxic effects potentiated by acetone. Such coexposure might make people more susceptible to the hepatotoxicity of these chemicals.

Renal Effects. No indication that acetone caused renal effects in humans was found in controlled studies of volunteers based on analysis of clinical chemistry parameters (blood urea nitrogen, uric acid) and urinalysis (DiVincenzo et al. 1973; Stewart et al. 1975). Furthermore, none of the located studies of workers or case reports documented renal effects.

The only indication that inhalation exposure to acetone causes renal effects in animals was the consistent finding of congestion or distention of renal tubules or glomeruli in guinea pigs exposed acutely to lethal levels of acetone (Specht et al. 1939). Rats exposed intermittently to 19,000 ppm for 18 weeks had no clinical or histological evidence of kidney damage (Bruckner and Peterson 1981b). Amyloidosis was observed in the kidneys of mice whose lumbo-sacral regions were painted twice weekly with an unspecified quantity of acetone for 12 months (Barr-Nea and Wolman 1977). The significance of the amyloidogenic effect of acetone to humans is unknown, since no clinical data

regarding amyloidosis in humans who come in frequent dermal contact with acetone, such as manicurists, are available.

No significant differences in kidney weight, BUN levels, or organic ion accumulation by renal slices were found in rats given 1,766 mg/kg/day acetone for 2 days compared with controls (Valentovic et al. 1992). Oral exposure of animals to acetone in other studies, however, has resulted in adverse renal effects, including degeneration of the apical microvilli of renal tubules in rats given a single oral dose of 871 mg/kg (Brown and Hewitt 1984); dose-related increased kidney weight in mice at ≥6,348 mg/kg/day in drinking water for 14 days (NTP 1991); increased kidney weight in female rats at ≥1,600 mg/kg/day and in male rats at 3,400 mg/kg/day, and a dose-related increased incidence and/or severity of nephropathy at ≥900 mg/kg/day in male rats, but not female rats, in drinking water for 13 weeks (NTP 1991); and increased kidney weight in female rats at 2500 mg/kg/day and in male rats at 2,500 mg/kg/day by gavage for 13 weeks (American Biogenics Corp. 1986). In addition, increased severity of renal proximal tubule degeneration and intracytoplasmic droplets of granules (hyaline droplets) in the proximal tubular epithelium showed a dose-related increase in males at ≥500 mg/kg/day and in females at 2,500 mg/kg/day (American Biogenics Corp. 1986). The kidney lesions seen in both the drinking water study and the gavage study may indicate that acetone enhanced the age-related nephropathy in rats. No kidney effects were observed in mice given acetone in the drinking water for 13 weeks (NTP 1991). Formate, one of the metabolites of acetone, may be responsible for the nephrotoxicity of acetone (NTP 1991).

Thus, species differences exist in susceptibility to acetone-induced kidney effects. Sex difference also exist; kidney weight increases in female rats at lower doses than in male rats, while histopathological lesions occur in male rats at lower doses than in females.

As in the liver, acetone also induced enzymes in microsomes prepared from kidneys of rats (Hong et al. 1987) and hamsters treated orally with acetone (Menicagli et al. 1990; Ueng et al. 1991). The increased kidney weights observed in rats and mice might also be associated with an increased amount of protein as a result of enzyme induction. Furthermore, acetone might potentiate the nephrotoxicity of other chemicals, such as chloroform, by increasing the rate of metabolism to reactive intermediate via enzyme induction (see Sections 2.3.5 and 2.6).

Since there is no indication that acetone causes kidney effects in humans or animals exposed by inhalation to nonlethal concentrations, exposure of people to acetone alone in ambient air, in air near hazardous waste sites, or at work sites probably poses little risk for the development of nephropathy. Whether levels in water or soil are high enough to increase kidney weight or cause other kidney effects is not known. The relevance of the acetone-enhanced age-related nephropathy seen in male rats treated orally with acetone (American Biogenics Corp. 1986; NTP 1991) to humans is not clear. The kidney lesions were seen in male rats, but not female rats or mice of either sex, in the NTP (1991) study. In the American Biogenics Corp. (1986) study, an increased severity of renal lesions was seen in female rats at the highest dose, but the increased severity was dose-related in male rats at lower doses and was associated with hyaline droplet formation. Hyaline droplet formation was not observed in the NTP (1991) study. The presence of hyaline droplets suggests that the renal effects observed in male rats may be associated with $\alpha_{2\mu}$-globulin syndrome, which appears to be specific for male rats, as this protein has not been found in female rats, mice, or humans (EPA 1991a). This low molecular weight protein has been implicated in the mechanism of chemically-induced renal toxicity and renal tumor formation in male rats for a number of chemicals. Thus, the renal pathology induced in male rats by acetone exposure may have no relevance to human health. The ability of acetone to induce microsomal enzymes may also pose a concern. Exposure to acetone alone is extremely unlikely in these scenarios, since many other chemicals are also present in air, soil, and water and at hazardous waste sites, and workers could be exposed to other chemicals with nephrotoxic effects that are potentiated by acetone. Such coexposure could make people more susceptible to the nephrotoxicity of these chemicals.

Dermal/Ocular Effects. Like many solvents, acetone is irritating to the skin and eyes. Dermal contact with vapors of acetone in air has not been reported to cause dermal effects in humans or animals. No studies were located regarding dermal or ocular effects in humans after oral exposure to acetone. Exposure of humans by direct contact of the skin with liquid acetone for 30 or 90 minutes has resulted in histological and ultrastructural degenerative changes in the epidermis (Lupulescu and Birmingham 1975, 1976; Lupulescu et al. 1972, 1973), and frequent dermal contact may cause contact dermatitis in sensitized humans (Tosti et al. 1988).

Oral exposure of animals to acetone in intermediate-duration studies did not cause any histological changes in the skin (NTP 1991), but dermal exposure of mice resulted in increased DNA synthesis, indicative of irritation (Iversen et al. 19SS), moderate hyperplasia of the epidermis (Iversen et al.

1981), hyperplasia, dermatitis, and hyperkeratosis (DePass et al. 1989); and amyloidosis in the skin (Barr-Nea and Wolman 1977). Hairless guinea pigs developed only mild erythema after direct application of acetone to the dorsal thorax (Taylor et al. 1993).

Like many solvents, acetone is irritating to the eyes. Humans exposed to acetone vapors in the air, either occupationally or in experimental studies, frequently complained of eye irritation (Matsushita et al. 1969a, 1969b; Nelson et al. 1943; Raleigh and McGee 1972; Ross 1973; Sallee and Sappington 1949). Concentrations of acetone in the air ≥100 ppm have been reported as irritating to the eyes of humans (Matsushita et al. 1969a). Eye irritation has also been observed in animals exposed to acetone vapors (Specht et al. 1939), and direct instillation of acetone into the eyes of rabbits has caused reversible cornea1 burns (Bolkova and Cejkova 1983), edema of mucous membranes (Larson et al.1956), severe eye necrosis (Carpenter and Smyth 1946; Smyth et al. 1962), and uveal melanocytic hyperplasia (Pe'er et al. 1992). Ocular effects have been observed in animals even after dermal exposure. Application of acetone to shaved skin of guinea pigs, but not rabbits for 3 or 6 weeks resulted in the development of cataracts (Rengstorff et al. 1972, 1976; Rengstorff and Khafagy 1985). Cataracts also occurred in guinea pigs after subcutaneous injection of acetone (3.8 mg/kg) 3 days/week for 3 weeks (Rengstorff et al. 1972). The difference in response between the guinea pigs and rabbits reflect species differences in susceptibility to the cataractogenic effects of acetone, as twice as much acetone was applied to rabbits than to guinea pigs. However, no cataracts or lens opacities were found in hairless guinea pigs to which acetone was applied to the skin for 6 months (Taylor et al. 1993). Genetic differences in susceptibility between hairless and normal guinea pigs was suggested to account for the different results, but was considered to be unlikely. Acetone produced a dose-related increased incidence of opacity in bovine cornea1 preparations *in vitro* (Gautheron et al. 1992). No cases of cataract formation in humans exposed to acetone by any route were located; therefore, the relevance of these findings to public health is not known. Histological and/or ophthalmoscopic examination of eyes of rats and mice did not reveal any ocular effects in intermediate-duration oral studies (American Biogenics Corp. 1986; NTP 1991).

Eye irritation is a concern for people exposed to acetone vapors occupationally, and splashing of liquid acetone into the eyes may cause cornea1 burns or necrosis, but concentrations of acetone in air in the general environment or near hazardous waste sites are probably not high enough to produce eye irritation. Dermal contact with soil containing high concentrations of acetone could possibly cause some dermal irritation, but acetone in water would probably be too dilute to irritate the buccal cavity.

The relevance of the observed cataract formation and amyloidosis in animals after dermal exposure to acetone to human health is not known.

Other Systemic Effects. No studies were located regarding other systemic effects in humans after inhalation or dermal exposure to acetone, but a case report of man who intentionally drank 200 mL pure acetone (2,241 mg/kg) described the subsequent development of diabetes-like symptoms, such as excessive thirst, polyuria, and hyperglycemia (Gitelson et al. 1966). In an acute study, treatment of fasted or fed rats by gavage with 3,214 mg/kg/day significantly reduced insulin-stimulated glucose oxidation in adipose tissue (Skutches et al. 1990). The reduction was greater in fasted rats than in fed rats. Acetone is a ketone so exposure can result in ketosis, and fasting can result in further ketosis through the production of ketone bodies in response to low fat intake. Diabetics with ketoacidosis produce more acetone endogenously than nondiabetic people (Fisher 1951; Reichard et al. 1986; Sulway and Malins 1970); therefore, the fact that exogenous acetone mimics some aspects of a diabetic-like condition is not surprising.

Most of the information regarding other systemic effects in animals after inhalation and oral exposure relates to body weight changes. Pregnant rats that were exposed to acetone by inhalation at 11,000 ppm during gestation had reduced body weights (NTP, 1988), but no effect on body weight of nonpregnant female rats exposed to 16,000 ppm for 2 weeks was found in another study (Goldberg et al., 1964). Furthermore, maternal body weights of mice were statistically significantly reduced on day 3 postpartum after treatment with 3,500 mg/kg/day acetone by gavage during gestation (EHRT 1987). It is possible that the condition of pregnancy made the rats and mice more susceptible to body weight reduction. Although body weight loss was observed in rats treated by gavage with lethal doses of acetone (Freeman and Hayes 1985), no indication of decreased body weight gain unrelated to decreased water consumption was found in animals exposed to acetone in drinking water (Furner et al. 1972; Hetu and Joly 1988; Ladefoged et al. 1989; NTP 1991; Valentovic et al. 1992). The only information on other systemic effects in animals exposed dermally to acetone was the finding of amyloidosis in the adrenals and pancreas of mice (Barr-Nea and Wolman 1977) and a transient weight loss of 60 g over a 2-week period in hairless guinea pigs to which acetone was applied to the skin for 6 months (Taylor et al. 1993). The relevance of these findings to human health is not known.

Exposure to acetone orally or by inhalation may result in ketosis, but whether concentrations of acetone in air, water, or soil in the general environment or at hazardous waste sites would result in

appreciable ketosis is not known. Occupational levels could be high enough to produce ketosis. Based on results of animal studies, pregnant women may be susceptible to body weight reduction if exposed to acetone, but it is unlikely that exposure levels in the environment or near hazardous waste sites would be high enough to cause body weight loss.

Immunological Effects. Information regarding immunological effects in humans after exposure to acetone is limited. Significantly increased white blood cell counts, increased eosinophil counts, and decreased phagocytic activity of neutrophils were found in volunteers exposed to 500 ppm for a single 6-hour exposure or intermittently for 6 days (Matsushita et al. 1969a, 1969b). A case report of a laboratory technician described the development of acute contact dermatitis from handling acetone 2 years after being treated with squaric acid dibutyl ester in acetone for patchy alopecia areata on her scalp (Tosti et al. 1988). This acetone sensitization is considered a rare complication of sensitizing therapies for alopecia areata. No studies were located regarding immunological effects in humans after oral exposure or in animals after inhalation, oral, or dermal exposure to acetone.

While hematological findings in humans may represent immunological effects, they have not been corroborated. Furthermore, other inhalation studies in humans that examined hematological end points did not find any abnormalities (DiVincenzo et al. 1973; Ott et al. 1983a, 1983c; Stewart et al. 1975). The development of contact dermatitis in the laboratory technician appears to be an isolated incident, but it is possible that acetone could produce dermatitis in sensitized humans who come in frequent dermal contact with acetone, such as laboratory workers.

Neurological Effects. No studies were located regarding neurological effects in animals after dermal exposure to acetone, but acetone exposure by the inhalation and oral routes has resulted in neurological effects, related to the narcotic effects of acetone, in both humans and animals. Patients who were exposed to acetone, mainly by inhalation, during and after the application of hip casts with acetone present in the setting fluid became comatose or collapsed (Chatterton and Elliott 1946; Fitzpatrick and Claire 1947; Harris and Jackson 1952; Renshaw and Mitchell 1956; Strong 1944). Some dermal exposure could have contributed to the total exposure. Headache, dizziness, weakness, difficulty speaking, and depression were experienced by a woman after an acetone-containing cast was applied (Pomerantz 1950). In addition, drowsiness, fretfulness, irritability, restlessness, uncoordinated hand movement, and nystagmus developed in patients after application of cast where exposure was considered to be mainly dermal (Hift and Pate1 1961). Neurological effects were commonly

experienced by workers exposed to acetone in the past. These include headache, lightheadedness, dizziness, unsteadiness, confusion, and unconsciousness (Raleigh and McGee 1972; Ross 1973), and have been observed at concentrations of acetone ≥901 ppm in the workplace. Neurobehavioral tests conducted in some of these workers did not reveal any effects of acetone on the parameters examined (Raleigh and McGee et al. 1972). Neurological and behavioral effects, such as, lack of energy and weakness, headache, delayed visual reaction time at 250 ppm for 6 hours or 6 hours/day for 6 days (Matsushita et al. 1969a, 1969b); subjective symptoms of tension, tiredness, and annoyance (Seeber and Kiesswetter 1991; Seeber et al. 1992); increases in response and the percent false negatives in auditory discrimination tests and increases in anger and hostility at 237 ppm for 4 hours (Dick et al. 1989); and increased visual evoked response at 1,250 ppm intermittently in complex protocol (Stewart et al. 1975) have been documented in volunteers tested under controlled laboratory conditions. Electroencephalography revealed no abnormalities (Stewart et al. 1975). In volunteers exposed to 21,049-84,194 ppm acetone for l-8 hours, the time to observation of signs of narcosis, loss of righting reflex, and loss of cornea1 reflex decreased as the exposure concentration increased (Haggard et al. 1944). It should be noted that these concentrations are higher than those resulting in unconsciousness in workers exposed for shorter durations.

Several case reports have described patients in unresponsive, lethargic, or comatose conditions after ingesting acetone (Gamis and Wasserman 1988; Ramu et al. 1978; Sakata et al. 1989), but most of these cases are confounded by coexposure to other possible narcotic agents. The lethargic and comatose conditions of these patients was, however, generally attributed to acetone poisoning. Deep coma developed in a man who intentionally ingested about 200 mL of pure acetone (about 2,241 mg/kg) (Gitelson et al. 1966). Six days after medical treatment, he was ambulatory but had a marked disturbance of gait, which had improved upon follow-up examination 2 months later.

Narcotic effects have been observed in animals acutely exposed to acetone vapors. Signs of narcosis, loss of righting reflex, and loss of cornea1 reflex were observed in rats exposed to concentrations of acetone ≥10,524 ppm (Haggard et al. 1944). Drowsiness, staggering, prostration, clonic movements of hind legs, and deep narcosis were observed in mice exposed to 16,839-84,194 ppm (Mashbitz et al. 1936). The time to observations of these signs decreased as the concentration of acetone increased. Narcosis as evidenced by decreased respiratory and heart rates, paralysis, and coma were observed in guinea pigs exposed to a lethal concentration (21,800 ppm) continuously for periods of 25 minutes ≤24 hours (Specht et al. 1939). The degree of narcosis increased as the exposure duration increased.

Severe narcosis was also observed in mice after a single 6-hour exposure to 11,000 ppm (NTP 1988). Neurobehavioral effects, indicative of narcosis, have been observed in rats (Bruckner and Peterson 1981a; Garcia et al. 1978; Geller et al. 1979b; Goldberg et al. 1964), mice (De Ceaurriz et al. 1984; Glowa and Dews 1987), and baboons (Geller et al. 1979a) acutely exposed to acetone vapors at exposure levels ≥150 ppm for baboons. A statistically significant decrease in absolute brain weight was observed, but no histopathological brain lesions, in rats exposed intermittently to 19,000 ppm in an intermediate-duration study (Bruckner and Peterson 1981a).

Acetone is also neurotoxic in animals after oral exposure. Death in mice and rats exposed acutely was preceded by prostration (EHRT 1987; Freeman and Hayes 1985). Weakness, depression, and unconsciousness were observed in rabbits dosed orally with 3,922-7,844 mg/kg, with the degree and time to onset dependent on dose (Walton et al. 1928). Incoordination, staggering, falling, tremors, delirium, prostration, and coma were observed in dogs and puppies at oral doses of 4,000-8,000 mg/kg (Albertoni 1884). In intermediate-duration oral studies, reduction in the nerve conduction velocity of rats exposed via drinking water at 650 mg/kg/day (Ladefoged et al. 1989) and excessive salivation in rats exposed by gavage at 2,500 mg/kg/day ≥27 days (American Biogenics Corp. 1986) were found. Absolute brain weight was lower in the gavaged rats at the 13-week sacrifice, but no histopathological lesions were found. No histopathological lesions were found in any of numerous central and peripheral nervous system tissues of rats given 732 mg/kg/day acetone in drinking water for 12 weeks (Spencer et al. 1978). However, no clinical or histopathological evidence was observed in rats or mice treated with higher doses for 13 weeks in the drinking water study (Dietz et al. 1991; NTP 1991), suggesting that the intermittent nature of *ad libitum* dosing via drinking water did not provide high enough doses, compared with the bolus nature of a gavage dose.

The mechanism by which acetone exerts its narcotic effects is not known, but based on the available data, it cannot be attributed to histologically observable changes in the brains of animals or to electroencephalographic changes in humans. As a solvent, however, acetone may interfere with the composition of membranes, altering their permeability to ions (Adams and Bayliss 1968). That acetone distributes to the brain was demonstrated in mice (Wigaeus et al. 1982) (see Section 2.3.2.1). The narcotic effects of acetone are generally reversible.

The narcotic effects of acetone represent a concern for public health, particularly in workers exposed occupationally, as occupational exposure in the case above exceeded the time-weighted average

standard of 750 ppm, but not the short-term exposure limit of 1,000 ppm (OSHA 1989). Narcotic effects are also of particular concern for accidental ingestion of acetone-containing household products, such as nail polish remover and liquid cement. Levels of acetone in ambient air, soil, or water, or levels present at hazardous waste sites are probably not high enough to cause narcosis or coma, but more subtle neurological and neurobehavioral effects may be of concern. Furthermore, acetone potentiates the neurotoxicity of 2,5-hexanedione by an unknown mechanism (Ladefoged et al. 1989; Lam et al. 1991; Strange et al. 1991) (see Section 2.6). Thus, coexposure to these neurotoxic ketones is of greater concern than exposure to either alone.

Reproductive Effects. No studies were located regarding reproductive effects in humans after oral or dermal exposure to acetone. Information regarding reproductive effects in humans after inhalation exposure is limited to reports of premature menstrual periods by 3 of 4 women exposed to 1,000 ppm acetone for 7.5 hours (Stewart et al. 1975), the lack of a statistically significant increased incidence of miscarriage in female laboratory workers exposed to a variety of solvents, including acetone (Axelsson et al. 1984), and an unreliable report of pregnancy complications in women factory workers exposed to acetone in Russia (Nizyaeva 1982).

Information regarding reproductive effects in animals after inhalation exposure is also limited. In an inhalation developmental study in rats and mice, no effects were found on the number of implants/litter, percent live pups/litter, or mean percent resorptions/litter (NTP 1988). No studies were located regarding reproductive effects in male animals, histological effects on reproductive organs of male or female animals, or the reproductive outcomes and other indices of reproductive toxicity in animals after inhalation exposure to acetone.

Reproductive effects (reduced reproductive index and increased duration of gestation) were found in pregnant mice exposed orally to 3,500 mg/kg/day during gestation (EHRT 1987). Intermediate-duration oral studies have shown that acetone produces reproductive effects in male Sprague-Dawley rats, but not male Wistar rats or male mice. Exposure of male Wistar rats to 1,071 mg/kg/day acetone in drinking water for 6 weeks did not affect successful mating to untreated females, number of pregnancies, number of fetuses, testicular weight, seminiferous tubule diameter, and testicular lesions (Larsen et al. 1991). However, statistically significantly increased relative testis weight (possibly due to decreased body weight), decreased sperm motility, caudal weight and epididymal weight, and increased incidences of abnormal sperm, but no histopathological testicular lesions, were found in male

Sprague-Dawley rats treated with 3,400 mg/kg/day acetone in drinking water for 13 weeks (NTP 1991). As discussed by NTP (1991), these effects on spermatogenesis are similar to those seen in alcoholics, and could be associated with the similarities in metabolism of acetone and ethanol. Vaginal cytology examinations of the female rats revealed no effects, and similar evaluation of male and female mice revealed no effects. While differences in susceptibility might account for the different results in Wistar and Sprague-Dawley rats, the Wistar rats were treated with a lower dose for a shorter duration. However, sex differences in rats, and species differences between male rats and male mice appear to exist.

No indication that dermal exposure of female mice results in histopathological effects in reproductive organs was found in an analysis of female SENCAR mice used as acetone controls in a skin painting study of formaldehyde (Ward et al. 1986).

The available data are too limited to predict whether exposure of humans to acetone under any conditions would result in reproductive effects. The finding of shortened menstrual cycles in women has not been corroborated.

Developmental Effects. No studies were located regarding developmental effects in humans after oral or dermal exposure to acetone. Information regarding developmental effects in humans after inhalation exposure is limited to a report that found no statistically significant increased incidence of miscarriage, perinatal death rate, or malformations of offspring in female laboratory workers exposed to a variety of solvents including acetone (Axelsson et al. 1984) and to an unreliable report of fetal death and low birth weight and length of neonates in female factory workers exposed to acetone in Russia (Nizyaeva 1982).

Developmental effects have been found in animals after inhalation and oral exposure. In rats exposed by inhalation to acetone during gestation, the only effect was a statistically significantly decreased mean male and female fetal body weight at 11,000 ppm, the same concentration at which dams displayed reduced body weight during gestation, reduced uterine weight, and reduced extragestational weight (NTP 1988). Mice similarly exposed were more susceptible than rats; at 6,600 ppm, statistically significantly increased incidence of late resorption, decreased fetal weight, and significantly increased incidence of reduced stemebral ossification were observed, along with increased absolute and relative liver weight of dams. Thus acetone produced fetotoxicity in rats and mice at concentrations

that were maternally toxic. Developmental toxicity was also found in mice after oral treatment of the dams with 3,500 mg/kg/day during gestation, such as reduced postnatal pup survival and reduced average weight of each live pup/litter on postpartum day 0 (EHRT 1987). Some of the dams treated at this dose displayed neurological signs of toxicity and died. Fetuses or pups were not examined for internal malformations or skeletal anomalies. No studies were located regarding developmental effects in animals after dermal exposure to acetone.

Acetone has been tested in attempts to develop *in vitro* methods for assessing developmental toxicity. Acetone at a concentration range of 0.l-1.0% (v/v) in the incubation medium had no effects on growth or malformations of 9.5 day postimplantation rat embryos or on growth and vascularization of the yolk sac (Schmid 1985). Negative results were also obtained for growth of 11 day mouse embryo limb buds at acetone concentrations of 10-100 mg/mL (Guntakatta et al. 1984). However, 10.5 day whole rat embryo cultures failed to grow and differentiate and eventually died at an acetone concentration of 2.5% (v/v) (Kitchin and Ebron 1984). A concentration of 0.5% acetone reduced yolk sac diameter and significantly increased the percentage of abnormal embryos (enlarged pericardium). At 0.1% acetone, abnormal brain development and edematous mandibular arches were also observed. Although results of studies conducted *in vitro* are difficult to extrapolate to *in vivo* exposure, acetone and its metabolites were found in fetuses from rats injected intravenously with 100 mg/kg acetone on gestational day 19, indicating transplacental transfer (Peinado et al. 1986).

Thus, acetone appears to be toxic to rat and mouse fetuses at maternally toxic inhalation concentrations and to developing neonatal mice at maternally toxic oral doses. Mice appear to be more susceptible than rats in *in vivo* studies. From limited information, acetone does not appear to be teratogenic in animals in *in vivo* experiments. Whether acetone would cause developmental toxicity in humans under any exposure conditions is not known, but concentrations and doses used in the animal studies were much higher than are likely to be experienced by humans.

Genotoxic Effects. No studies were located regarding the genotoxicity of acetone in humans or animals after inhalation, oral, or dermal exposure. The results of *in vivo* studies conducted by intraperitoneal injection are summarized in Table 2-4. Negative results were obtained in the micronucleus test in Chinese hamsters (Basler 1986) and for cell transformation in fetal cells from pregnant hamsters (Quarles et al. 1979a, 1979b). In addition, tests for gene mutation in silk worms exposed by an unspecified route were negative (Kawachi et al. 1980). Acetone has been tested for

TABLE 2-4. Genotoxicity of Acetone *In Vivo*

Species (test system)	End point	Results	Reference
Chinese hamsters	Micronuclei in erythroycytes	—	Basler 1986
Pregnant hamsters	Cell transformation in fetal cells	—	Quarles et al. 1979a, 1979b
Silk worms	Gene mutation	—	Kawachi et al. 1980

— = negative result

genotoxicity in numerous assays *in vitro*, and results of representative studies are summarized in Table 2-5. Results were negative for reverse mutation in all tested strains of *Salmonella typhimurium* (DeFlora 1981; DeFlora et al. 1984; Ishidate et al. 1984; Kawachi et al. 1980; McCann et al. 1975; Yamaguchi 1985), in the ret assay in *Bacillus subtilis* (Kawachi et al. 1980) with and without metabolic activation, and for prophage induction (De Marini et al. 1991; Rossman et al. 1991; Vasavada and Padayatty 1981) and DNA binding (Kubinski et al. 1981) in *Escherichia coli*. However, with enzyme-generated triplet acetone (Menck et al. 1986) or photo-generated triplet acetone (Rahn et al. 1974), results were positive for DNA damage in *E. coli* lambda prophage (Menck et al. 1986) and DNA chain breaks and thymidine dimers in *E. coli* (Rahn et al. 1974). Triplet acetone, which is electronically excited in one of the methyl groups, can be generated by endogenous enzymatic reactions or by photochemical reactions (Menck et al. 1986). Thus, the relevance of the DNA damage induced by triplet acetone to human exposure scenarios to acetone is questionable. Results were mixed in fungi (Abbandandolo et al. 1980; Albertini 1991; Zimmermann 1983; Zimmermann et al. 1984, 1985), and results for gene mutation were negative in *Arubidopsis thaliana* seeds (Gichner and Veleminsky 1987). Results have usually been negative in assays for cell transformation, chromosomal aberrations, sister chromatid exchange, colony formation inhibition, and gene mutation in cultured animal cells, and for sister chromatid exchange and unscheduled DNA synthesis in cultured human fibroblasts and skin epithelial cells (see Table 2-5). However, some positive results were obtained for chromosomal aberrations in Chinese hamster fibroblasts (Ishidate et al. 1984) and hamster lung fibroblasts (Kawachi et al. 1980). Acetone also inhibited metabolic cooperation (intracellular communication) in Chinese hamster V79 cells (Chen et al. 1984) and produced chromosome malsegregation in porcine brain tubulin (Albertini et al. 1988). The overwhelming evidence is that acetone is, at best, weakly genotoxic. Genotoxicity assay conditions vary widely, and probably account for different results in similar cell systems.

Because of its solvent properties and mostly lack of genotoxic effects, acetone is often used as a solvent for testing the genotoxicity of other chemicals and as the solvent control in these assays (Nestmann et al. 1985; Norppa 1981; Norppa et al. 1981). Acetone was used as the solvent control to study the promoting activity of 12-O-tetradecanoylphorbol 13-acetate in a 2-stage cell transformation assay in the BALB/3T3 cell line (Sakai and Sato 1989). No indication that acetone promoted the transforming activity of nine known genotoxic and carcinogenic chemicals was found.

TABLE 2-5. Genotoxicity of Acetone *In Vitro*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
Prokaryotic organisms:				
Salmonella typhimurium	Reverse mutation			DeFlora 1981; DeFlora et al. 1984
TA98		No data	—	
TA100		No data	—	
TA1535		No data	—	
TA1537		No data	—	
TA1538		No data	—	
S. typhimurium	Reverse mutation			Yamaguchi 1985
TA100		—	—	
TA98		—	—	
S. typhimurium	Reverse mutation			Ishidate et al. 1984
TA92		—	No data	
TA94		—	No data	
TA98		—	No data	
TA100		—	No data	
TA1535		—	No data	
TA1537		—	No data	
S. typhimurium	Reverse mutation			McCann et al. 1975
TA98		—	No data	
TA100		—	No data	
TA1535		—	No data	
TA1537		—	No data	
S. typhimurium	Reverse mutation			Kawachi et al. 1980
TA100		—	—	
TA98		—	—	

TABLE 2-5. Genotoxicity of Acetone *In Vitro (continued)*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
Escherichia coli (WP2$_s$(λ)	Prophage λ induction	—	—	DeMarini et al. 1991; Rossman et al. 1991
E. coli (λ phage)	DNA damage	+[a]	No data	Menck et al. 1986
E. coli CR63 colitis bacteriophage	Transfection (induction of phage)	—	—	Vasavada and Padayatty 1981
E. coli B(3)T	DNA chain breaks	+[a]	No data	Rahn et al. 1974
	thymidine dimers	+[a]	No data	
E. coli	DNA binding	—	—	Kubinski et al. 1981
Bacillus subtilis	Rec assay	—	—	Kawachi et al. 1980
Eukaryotic organisms:				
Fungi:				
Saccharomyces cerevisiae D61.M	Aneuploidy	No data	+	Zimmermann 1983; Zimmerman et al. 1984, 1985
S. cerevisiae D61.M	Mitotic chromosome malsegregation	No data	+	Albertini 1991
S. cerevisiae D61.M	Increased frequency of resistant colonies	No data	—	Albertini 1991
Saccharomyces pombe	Forward mutation	—	—	Abbandandolo et al. 1980
Plants:				
Arabidopsis thaliana seeds	Gene mutation	No data	—	Gichner and Veleminsky 1987
Mammalian cells:				
Syrian hamster embryo cells	Cell transformation	No data	—	Di Paolo et al. 1969
Syrian hamster embryo cells	Cell transformation	No data	—	Pienta 1980
Chinese hamster ovary cells	Chromosomal aberrations	—	—	Tates and Kriek 1981
Chinese hamster fibroblasts	Chromosomal aberrations	No data	+	Ishidate et al. 1984

TABLE 2-5. Genotoxicity of Acetone *In Vitro (continued)*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
Hamster lung fibroblasts	Chromosomal aberrations	No data	+	Kawachi et al. 1980
Chinese hamster ovary cells	Sister chromatid exchange	—	—	Tates and Kriek 1981
Chinese hamster cells	Sister chromatid exchange	No data	—	Abe and Sasaki 1982
Hamster lung fibroblasts	Sister chromatid exchange	No data	—	Kawachi et al. 1980
Chinese hamster V79 cells	Inhibition of metabolic cooperation (intracellular communication)	No data	+	Chen et al. 1984
Chinese hamster V79 cells	Inhibition of colony formation	No data	—	Chen et al. 1984
AKR leukemia virus-infected mouse embryo cells	Cell transformation	No data	—	Rhim et al. 1974
Mouse lymphoma TK cells	Gene mutation	No data	—	Amacher et al. 1980
Rat embryo culture	Cell transformation	No data	—	Freeman et al. 1973
Rat embryo cells infected with Rauscher leukemia virus	Cell transformation; ouabain resistance	— —	— —	Mishra et al. 1978
Porcine brain tubulin	Chromosome malsegregation	No data	+	Albertini et al. 1988
Human fibroblasts	Sister chromatid exchange	No data	—	Kawachi et al. 1980
Human fibroblasts	Sister chromatid exchange	No data	—	Abe and Sasaki 1982
Human skin epithelial cells	unscheduled DNA synthesis	No data	—	Lake et al. 1978

[a]Activation to triplet state

— = negative result; + = positive result; DNA = deoxyribonucleic acid

The weight of evidence for the genotoxicity indicates that acetone poses little threat for genotoxic effects in humans exposed to acetone under any conditions.

Cancer. No studies were located regarding cancer in humans after oral or dermal exposure. A retrospective mortality study of workers exposed to time-weighted-average acetone concentrations of 380-1,070 ppm at a cellulose fiber plant where acetone was used as the only solvent found no significant excess risk of death from any cause, including malignant neoplasm, compared with rates for the U.S. general population (Ott et al. 1983a, 1983b).

No studies were located regarding cancer in animals after inhalation or oral exposure to acetone. In an analysis of the histopathology in female SENCAR mice used as acetone controls in a skin painting study of formaldehyde, no neoplastic lesions associated with acetone exposure were found (Ward et al. 1986). Furthermore, acetone was negative as a tumor promoter for formaldehyde. In addition, no evidence was found that acetone was a skin carcinogen when used as a negative control for organosilanes (DePass et al. 1989) or flame retardants (Van Duuren et al. 1978) in skin painting studies in mice. Acetone was also negative as a tumor initiator (Roe et al. 1972) and as a tumor promoter for 7,12-dimethylbenz[a]anthracene (Roe et al. 1972; Van Duuren et al. 1971; Weiss et al. 1986).

EPA has classified acetone in Group D, that is, not classifiable as to human carcinogenicity due to the lack of data concerning carcinogenicity in humans and animals (IRIS 1992). Acetone has not been classified by the National Toxicology Program (NTP) or the International Agency for Research on Cancer (IARC).

2.5 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NASLNRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s), or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally

the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to acetone are discussed in Section 2.5.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by acetone are discussed in Section 2.5.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.7, "Populations That Are Unusually Susceptible."

2.5.1 Biomarkers Used to Identify or Quantify Exposure to Acetone

Acetone concentrations in expired air, blood, and urine have been monitored in a number of studies of humans exposed to acetone in the workplace as well as in controlled laboratory situations, and correlations with exposure levels have been found. However, acetone is cleared from breath, urine, and blood within 1-3 days, so these methods are useful for monitoring only for recent exposure to acetone. In addition, these methods can be used to detect or confirm relatively high exposure to

acetone, such as what might occur in the workplace or from accidental ingestion, but they cannot be used to detect exposure in the general population at levels reasonably likely to occur outside the workplace. The detection of acetone odor in the breath can alert a physician that a nondiabetic patient has been exposed to acetone (Harris and Jackson 1952; Strong 1944). It should be noted that exposure to other chemicals that are metabolized to acetone, such as isopropyl alcohol, could also lead to elevated blood, expired air, or urinary levels of acetone. Levels of endogenous acetone can fluctuate greatly due to normal diurnal variations (Wildenhoff 1972). In addition, physical exercise (Koeslag et al. 1980); nutritional status and fasting (Jones 1987; Kundu et al. 1993; Levy et al. 1973; Lewis et al. 1977; Neiman et al. 1987; Reichard et al. 1979; Rooth and Carlstrom 1970; Williamson and Whitelaw 1978); trauma (Smith et al. 1975); and pregnancy and lactation (Bruss 1989; Paterson et al. 1967) place high energy demands upon the body, resulting in increased fatty acid utilization and higher than average blood levels of acetone. Diabetes (Kobayashi et al. 1983; Levey et al. 1964; Reichard et al. 1986; Rooth 1967; Rooth and Ostenson 1966) and alcohol use (Phillips et al. 1989; Tsukamoto et al. 1991) may result in high levels of endogenous acetone. Infants and young children typically have higher acetone in their blood than adults due to their higher energy expenditure (Peden 1964). These factors and physiological states can complicate measuring acetone levels in blood, breath, and urine for biomonitoring purposes.

In a group of 115 workers, alveolar air samples obtained during the workshift were collected at the same time as breathing zone acetone concentrations (Brugnone et al. 1980). The mean ratio of alveolar air acetone and breathing zone acetone was 0.288. Correlations were high between alveolar air concentrations and breathing zone concentrations. Because the alveolar air samples and breathing zone concentrations were collected at the same time, and since the equilibration of alveolar air with environmental air requires some time, the alveolar samples might not necessarily reflect the environmental concentration. Similar results were obtained in a group of 20 workers in a shoe factory in which the mean environmental air concentrations ranged from 10 to 12 ppm at four sampling times (Brugnone et al. 1978). The mean alveolar concentrations ranged from 2.75 to 3.75 ppm at three sampling times during the workshift. The correlation was good between workroom air concentration and alveolar air concentration, indicating that alveolar air concentrations of acetone are useful for monitoring concurrent occupational exposure to acetone. In a group of 110 male workers exposed to acetone for an average of 14.9 years, alveolar air samples were collected before work and at the end of work on 2 consecutive days (Fujino et al. 1992). The breathing zone concentrations of acetone were measured for each individual with personal monitors and ranged from 0 to about 1,200 ppm, with

most concentrations between 100 and 500 ppm. The average concentration of acetone in alveolar air before exposure on the first day was 2.95 ppm. Alveolar air concentrations at the end of the workday (range of about 20 to 300 ppm, average not reported) correlated strongly with exposure concentrations (r=0.65). It was estimated that the alveolar air concentrations corresponding to the ACGIH threshold limit value (TLV) of 750 ppm and to the Japan Association of Industrial Health acceptable concentration of 200 ppm were 177 and 56.2 ppm, respectively.

Expired air concentrations of acetone have also been studied in volunteers exposed to acetone in controlled laboratory situations. In 11 men and 11 women exposed to 237 ppm acetone for 2 or 4 hours, alveolar breath samples collected immediately after exposure contained mean levels of acetone of 21.5 ppm in those exposed for 2 hours and 25.8 ppm in those exposed for 4 hours (Dick et al. 1989). The alveolar air concentrations of acetone dropped to 12.8 ppm by 90 minutes after the 4-hour exposure and to background levels of 0.6 ppm by 20 hours postexposure. In humans exposed to acetone at ≤1,250 ppm for ≤7.5 hours/day in a complex protocol for ≤6 weeks, the rate of respiratory excretion was a function of the duration, and the concentration of acetone in breath after exposure was directly related to the time-average concentration during exposure, with constant duration (Stewart et al. 1975). The length of time after exposure in which acetone could be detected in breath was related to the magnitude of exposure; acetone was still readily detectable 16 hours after exposure to 1,000 or 1,250 ppm for 7.5 hours. Therefore, breath analysis can be used as a rapid method to estimate the magnitude of recent acetone exposure, but has limited usefulness for more remote exposure because elimination in expired air is generally complete within 1 day.

As discussed in Section 2.3.4.1, the level and nature of physical activity, the exposure concentration, the duration of exposure, and gender can influence the rate and amount of acetone elimination in the breath (DiVincenzo et al. 1973; Nomiyama and Nomiyama 1974a, 1974b; Pezzagno et al. 1986; Wigaeus et al. 1981). In general more acetone is expired faster following exposure to high concentrations than to low concentrations (DiVincenzo et al. 1973). Doubling the duration of exposure almost doubles the total amount of acetone expired. Exercise during exposure eliminates nearly twice the amount in expired air compared with exposure to the same concentration at rest, due to increased uptake from increased pulmonary ventilation. Furthermore, exercising at stepwise increments in workload during exposure results in greater respiratory elimination than exercising at a constant workload (Wigaeus et al. 1981). Women appeared to expire acetone more slowly than men,

but the total expired by women was not statistically significantly different than the total expired by men (Nomiyama and Nomiyama 1974a, 1974b).

Acetone is mainly excreted in the expired air after oral exposure as well as after inhalation exposure (see Section 2.3.4.2). Since urinary clearance of acetone is minimal, the calculated clearance of acetone from the lungs was 29 ml/minute or 0.39 ml/minute/kg for a patient who ingested nail polish remover using an average minute ventilation of 9.65 L/minute based on the patient's age, weight, and sex (Ramu et al. 1978). With a volume of distribution of 0.82 L/kg, the calculated half-life was 25 hours.

Monitoring of expired air for acetone exposure should take into consideration background levels of acetone, since acetone is produced endogenously in the body, especially during fasting and in diabetics. In addition, the ingestion of ethanol can influence the breath levels of acetone. Endogenous levels of acetone in normal humans averaged 0.56 ppm (Phillips and Greenberg 1987). Endogenous levels of acetone in alveolar air in a group of volunteers in an experimental study averaged 0.108 ppm (Wigaeus et al. 1981). In healthy men who had fasted for 12 hours, the breath acetone levels ranged from 0.96 to 1.7 ppm (Jones 1987). Fasting for 36 hours resulted in average acetone breath levels of 14-66 ppm. However, in fasting men who ingested 0.25 g/kg of ethanol, the breath acetone levels decreased by 40% after a 12-hour fast and by 18% after a 36-hour fast (Jones 1988).

Acetone is metabolized to carbon dioxide (see Section 2.3.3), which is eliminated in expired air (see Section 2.3.4). However, since carbon dioxide is the main constituent of normal respired air, expired carbon dioxide has not been monitored to determine acetone exposure.

Although unchanged acetone is excreted mainly by the lungs, urinary levels are sufficiently high for monitoring purposes. In a group of 104 workers employed at factories in which breathing zone levels of acetone ranged from <242 to <1,452 ppm, urine was collected before the workshift and 4 hours later (Pezzagno et al. 1986). A close correlation was found between the time-weighted average workroom concentration and the urinary concentration of acetone. The equation obtained was: urinary concentration (μmol/L) = 0.033 x time-weighted average environmental concentration (umol/m^3) = 0.005 (r=0.94, n=104). In another study of 28 workers, personal breathing zone monitoring revealed wide variation depending on the type of job and ranged from <1 to 30 ppm (Kawai et al. 1990a). Results of stationary monitoring revealed workroom concentrations ranging from

1.4 to 16.2 ppm. Urine was collected at the end of the workshift, and acetone was detected in the urine of all the workers. The concentration of acetone in urine was linearly correlated with the breathing zone concentration as follows: acetone in urine (mg/L) = 0.10 + 0.40 x breathing zone concentration (ppm) (r=0.90, p<O.01). Therefore, urinary levels of acetone are useful for monitoring occupational exposure. In another study, postshift urinary levels of acetone in 45 workers exposed to 0-70 ppm acetone ranged from 0 to 17.5 mg/L (Kawai et al. 1992). The background urinary level of acetone in nonexposed subjects was 1.5 mg/L. Acetone levels in preshift urine samples were significantly higher than background levels when acetone exposure on the previous day was >15 ppm, but there was no significant difference between background urine levels and preshift urine levels when acetone exposure on the previous day was ≤15 ppm. In a group of 110 males workers exposed to acetone for an average of 14.9 years, urine samples were collected before work and at the end of work for 2 consecutive days (Fujino et al. 1992). The breathing zone concentrations of acetone were measured for each individual with personal monitors and ranged from 0 to about 1,200 ppm, with most concentrations between 100 and 500 ppm. The average urinary concentration before exposure on the first day was 2.44 mg/L. Urinary levels at the end of the workshift (range of about 5 to 150 ppm, average not reported) correlated strongly with exposure concentration (r=0.71). It was estimated that the urinary concentrations corresponding to the ACGIH TLV of 750 ppm and to the Japan Association of Industrial Health acceptable concentration of 200 ppm were 76.6 and 21.6 mg/L, respectively.

Acetone has also been detected in the urine of 15 subjects exposed to acetone under controlled laboratory conditions. In volunteers exposed to 23-208 ppm for 2-4 hours, the urinary concentrations of acetone immediately after exposure ranged from 18.8 to 155.2 μmol/L and displayed statistically significant linear relationships with the exposure concentrations (Pezzagno et al. 1986). The regression equation for subjects exposed for 2 hours at rest was: acetone in urine (μmol/L) = 0.0125 x environmental concentration (μmol/m^3) + 5.87 (r=0.98, n=5). For subjects exposed for 4 hours at rest the equation was: acetone in urine (μmol/L) = environmental concentration (μmol/m^3) + 6.97 (r=0.96, n=5). For the subjects exposed for 2 hours with exercise, the equation was: acetone in urine (pmol/L) = environmental concentration (μmol/m^3) - 4.52 (r=0.99, n=5). At 4 hours after exposure, the urine concentration increased to 120% of that measured immediately after exposure, then fell to 65% at 7 hours, 45% at 9 hours, 35% at 12 hours, and 15% at 20 hours. Urinary acetone was completely cleared within 20 hours from subjects exposed to 242 or 542 ppm for 2 hours, regardless of whether or not they had exercised during exposure (Wigaeus et al. 1981). In a group of subjects exposed to acetone vapors for about 6 hours, urinary levels of acetone peaked within the first hour after exposure

to 1.8 mg/dL at 250 ppm, 2.9 mg/dL at 500 ppm, and 5.3 mg/dL at 1,000 ppm and declined rapidly after exposure to control levels within 24, 32, and 48 hours, respectively (Matsushita et al. 1969b). In subjects exposed to 250 ppm for 6 hours/day for 6 days either at rest or during exercise, the urinary levels declined to normal by the next morning each day and within 48 hours after the last exposure day, regardless of whether or not they had exercised (Matsushita et al. 1969a). However, in subjects exposed to 500 ppm 6 hours/day for 6 days, the level of acetone in the urine fell each day, but not to background levels. After the last day of exposure, urinary levels declined to background levels within 3 days. Background urinary levels of acetone in these subjects were about 0.1 mg/dL. Therefore, the rate of urinary clearance is dependent on the magnitude of exposure.

Acetone can also be detected in urine after oral exposure. In a patient who was admitted to the hospital in a comatose condition after ingesting sake and liquid cement containing 18% acetone (231 mg/kg), urinary clearance of acetone was followed, but after he had been subjected to gastric lavage (Sakata et al. 1989). Urine levels of acetone decreased gradually from 123 μg/mL at 5 hours after ingestion to about 61 μg/mL at 19 hours. Acetone then disappeared more rapidly from the urine.

Formic acid was detected in the urine of rats collected for 7 days after exposure to 62,000 ppm acetone in air, and was excreted at a rate of 344 μg formic acid/hour, compared with controls that excreted formic acid at a rate of 144 μg/hour (Hallier et al. 1981). The authors concluded that the low rate of formic acid excretion by rats suggests that 24 hours is an insufficient period of time for following formic acid excretion in order to biomonitor acetone exposure in humans.

Blood levels of acetone can also be useful for exposure monitoring, but blood sampling is less desirable because it is invasive. In a group of 110 males workers exposed to acetone for an average of 14.9 years, blood samples were collected before work on the first day and at the end of work on the second day (Fujino et al. 1992). The breathing zone concentrations of acetone were measured for each individual with personal monitors and ranged from 0 to about 1,200 ppm, with most concentrations between 100 and 500 ppm. The average blood concentration before exposure on the first day was 3.80 mg/L. Blood levels at the end of the workshift (range of about 2 to 225 mg/L, average not reported) correlated strongly with exposure concentration (r=0.65). It was estimated that the blood concentrations corresponding to the ACGIH TLV of 750 ppm and to the Japan Association of Industrial Health acceptable concentration of 200 ppm were 118 and 41.4 mg/L, respectively. Subjects exposed to 100 or 500 ppm for 2 or 4 hours had a blood acetone clearance half-life of 3 hours

(DiVincenzo et al. 1973). The rate of blood elimination was constant regardless of blood acetone concentration. In volunteers exposed to 237 ppm acetone, blood levels of acetone averaged 2.0 μg/mL preexposure, 9.0 μg/mL after 2 hours of exposure, 15.3 μg/mL after 4 hours of exposure, 11.9 μg/mL at 90 minutes postexposure, and 1.5 μg/mL at 20 hours postexposure (Dick et al. 1989). Therefore, elimination of acetone from blood was complete 20 hours after exposure. Results were similar for subjects exposed to acetone vapors for =6 hours (Matsushita et al. 1969b). Maximum blood levels of acetone achieved and blood clearance of acetone were exposure concentration-related, but not in direct proportion. At an exposure level of 250 ppm, the maximum blood level was 2 mg/dL and declined to background level within 24 hours. At an exposure level of 500 ppm, the maximum blood level was 4.7 mg/dL and declined to background level within 32 hours. At an exposure level of 1,000 ppm, the maximum blood level of 6.0 mg/dL declined to background level within 48 hours. In subjects exposed 6 hours/day for 6 days, maximum blood levels on each day were similar to those seen in the subject exposed only 1 day (Matsushita et al. 1969a). Blood levels declined to background levels on the morning after exposure on each day when the exposure concentration was 250 ppm. With an exposure concentration of 500 ppm, however, the blood levels declined each day, but not to background levels. As with urinary clearance, blood clearance of acetone at the end of the 6-day exposure period declined to background within 2 days at 250 ppm and within 3 days at 500 ppm. Background blood levels of acetone in these subjects were about 0.1 mg/dL. In subjects exposed to 242 or 542 ppm for 2 hours, the arterial blood concentration 1 hour after exposure plotted as a function of total uptake gave a linear relationship, indicating that an arterialized capillary sample during or after exposure may be useful for exposure monitoring (Wigaeus et al. 1981). In humans exposed to acetone ≤1,250 ppm for ≤7.5 hours/day in a complex protocol for ≤6 weeks, the concentration of acetone in venous blood was directly related to the vapor concentration and duration of exposure and inversely related to the time elapsed following exposure (Stewart et al. 1975). Using a physiologically-based pharmacological model, Leung and Paustenbach (1988) calculated a biological exposure index of 35 mg acetone/L blood for occupational exposure. The authors reported a background acetone blood level of 2 mg/L. This value is in agreement with normal background levels determined in other studies: 0.016 mM (0.93 mgn) (Gavin0 et al. 1986); 0.03 mmol/L (1.74 mg/L) (Trotter et al. 1971); 2100 ppb (2.1 mg/L) (Ashley et al. 1992).

Similar rates of blood acetone clearance occur after oral exposure. In a patient admitted to the hospital in a comatose condition after ingesting liquid cement containing 18% acetone (231 mg/kg), the plasma level of acetone was 110 μg/rnL at 5 hours after ingestion and declined to 65 μg/rnL at

18 hours, to 60 μg/mL at 20 hours, and to <5 μg/mL at 48 hours (Sakata et al. 1989). The gastric contents of a patient were analyzed using infrared spectrophotometry and found to contain 1 mL acetone/100 mL (Fastlich 1976). This analytical method was developed to detect volatile solvents in gastric contents due to accidental ingestion of these solvents.

Acetone has been identified in breast milk of lactating women (Pellizzari et al. 1982). According to the authors, mother's milk is an attractive medium for biomonitoring purposes because sample collection is reasonably straight-forward, milk contains a high amount of fat, so that fat-soluble pollutants may be found at higher concentrations in milk than in blood or urine, large volumes are easily collected, and the population of nursing mothers is relatively large. A disadvantage is the fact that only young to middle-age females are nursing, making extrapolation to the general population difficult.

As discussed in Section 2.9.3, an abstract indicated that acetone increased the levels of DNA adducts in rats exposed via drinking water (Cunningham and Gold 1992).

2.5.2 Biomarkers Used to Characterize Effects Caused by Acetone

The most consistently observed effect of acetone exposure in animals is the induction of microsomal enzymes, particularly of cytochrome P-450IIEl (see Sections 2.2.2.2, 2.35, and 2.6). The enzyme induction has been associated with increased liver weights and hepatocellular hypertrophy due to the increased protein content (NTP 1991). Acetone itself is only moderately toxic to the liver of animals, as most studies have found no clinical or histological evidence of liver damage. However, increased levels of serum alanine aminotransferase, which constitutes clinical evidence of liver damage, have been found in rats in one study (American Biogenics Corp. 1986). Cytochrome P-450IIEl is associated with the metabolism of acetone itself, but acetone is not metabolized to toxic intermediates (see Section 2.3). However, the induction of this enzyme by acetone is the mechanism by which acetone potentiates the hepatotoxicity, nephrotoxicity, genotoxicity, and perhaps the reproductive and hematological toxicity of other chemicals (see Section 2.6). Cytochrome P-450IIEl can be induced by a variety of other factors, such as exposure to ethanol, fasting, and experimental diabetes (Johansson et al. 1986; Puccini et al. 1990); therefore, the induction is not specific to acetone. Moreover, the detection of enzyme induction might require invasive methods, such as liver biopsy. As discussed in Section 2.9.3, an *in vivo* probe is being developed to identify human populations with elevated levels

of cytochrome P-45011El. These persons are thought to be at greater risk for developing the carcinogenicities and cytotoxicities related to P-45011El activity.

Exposure of animals to acetone has resulted in degeneration of apical microvilli in renal tubules (Brown and Hewitt 1984) and enhancement of nephropathy commonly seen in aging rats (NTP 1991), but these effects have not been associated with increased levels of blood urea nitrogen.

As is typical of many organic solvents, acetone is irritating to respiratory mucosa, the skin, and eyes. Acetone exposure can also result in such nonspecific narcotic effects as headache, dizziness, lightheadedness, confusion, unconsciousness (DiVincenzo et al. 1973; Matsushita et al. 1969a, 1969b; Nelson et al. 1943, Raleigh and McGee 1972; Ross 1973), some neurobehavioral and hematological effects (Dick et al. 1989; Matsushita et al. 1969a; Stewart et al. 1975), and perhaps menstrual disorders (Stewart et al. 1975). In addition, patients who had hip casts applied with acetone as the setting fluid became nauseous, vomited blood, and had a strong odor of acetone in the breath. These symptoms were associated with the subsequent development of unconsciousness (Harris and Jackson 1952; Strong 1944). The detection of a strong acetone odor on the breath and nausea could alert physicians to the development of more serious sequelae, such as gastrointestinal hemorrhage and narcosis.

Since acetone is a ketone, acetone exposure can lead to ketosis and other diabetes-like symptoms in humans (Gitelson et al. 1966) and to reduced insulin-stimulated glucose oxidation in animals (Skutches et al. 1990). Again the detection of a strong odor of acetone on the breath, or high levels of acetone in blood or urine can alert physicians to these effects.

In male rats, acetone exposure resulted in anemia as detected by hematological parameters (American Biogenics Corp. 1986; NTP 1991), and in increased testis weight, decreased sperm motility, caudal and epididymal weight, and increased incidences of abnormal sperm (NTP 1991). Hematological tests and tests for sperm motility and abnormalities could be used to screen humans for possible hematological and fertility effects.

Dermal exposure of humans to acetone irritated the skin, which when examined by light and electron microscopy, showed signs of degenerative changes in the epidermis (Lupulescu and Birmingham 1976; Lupulescu et al. 1972, 1973). Decreased protein synthesis was also found (Lupulescu and Birmingham

1975). Overt signs of skin irritation could alert physicians to possible degenerative changes. Allergic reactions to acetone can be detected by patch testing (Tosti et al. 1988).

As most of the effects of acetone are not specific to acetone, there is no reliable biomarker of effect that can be used to detect or screen for possible effects from exposure to acetone at levels reasonably likely to occur outside the workplace or from accidental ingestion.

For more detailed discussion of the effects caused by acetone see Section 2.2. Further information on biomarkers for specific organ systems (immune, renal, hepatic) can be found in ATSDR/CDC (1990) and on neurological effects in OTA (1990).

2.6 INTERACTIONS WITH OTHER SUBSTANCES

While acetone by itself is only moderately toxic, it potentiates the toxicity of a variety of chemicals, including halogenated alkanes and alkenes, benzene, dichlorobenzene, ethanol, 2,5-hexanedione, nitrosamines, acetonitrile, and acetaminophen. By far, the most extensively studied interactions are those with carbon tetrachloride and chloroform. In most of the interactions discussed below, acetone exerts its potentiating effect by inducing microsomal mixed function oxidases, in particular cytochrome P-45OIIEl and P-450IIEl-dependent enzyme activities, that bioactivate the other chemicals to reactive metabolites.

Halogenated Alkanes and Alkenes. No studies were located regarding the effects of coexposure of humans to acetone and carbon tetrachloride. However, acetone, a metabolite of isopropyl alcohol, was implicated in a study of workers in a isopropyl alcohol packaging plant who became ill after accidental exposure to carbon tetrachloride (Folland et al. 1976). Fourteen workers became ill (nausea, vomiting, headache, and weakness or abdominal pain, dizziness, diarrhea, and blurred vision). Workers in closer proximity to isopropyl alcohol were especially affected. Renal failure and hepatitis developed in 4 of the workers with closer proximity to isopropyl alcohol. Expired air samples taken subsequently from workers during isopropyl alcohol bottling revealed strikingly elevated levels of acetone (mean = 19 ppm in workers on the bottling line and 7.5 ppm in more remote workers). The blood acetone levels were 3-30 times higher than the normal range. Thus it appeared that isopropyl alcohol, by way of acetone, predisposed the workers to the hepatotoxicity and renal toxicity induced by carbon tetrachloride.

The potentiation of carbon tetrachloride-induced hepatotoxicity and renal toxicity by acetone has been well documented in rats. Pretreatment of rats by gavage with acetone enhanced the hepatotoxicity of carbon tetrachloride, as evidenced by the statistically significantly increased relative liver weights, increased severity of histopathological lesions (necrosis, hepatocellular swelling, lipid droplets), activities of serum alanine aminotransferase and ornithine carbamoyltransferase, the serum concentration of bilirubin, and/or the liver concentration of triglycerides compared with the liver toxicity induced by carbon tetrachloride alone in several studies (Charbonneau et al. 1985; 1986a, 1986c; 1988; 1991; Plaa et al. 1973, 1982; Plaa and Traiger 1972; Traiger and Plaa 1973, 1974). Acetone treatment alone had no effect on these parameters. The potentiation increased in a doserelated manner at single doses of acetone ≥0.25 mL/kg (≥196 mg/kg); doses of ≤0.10 mL/kg (≤78 mg/kg) are ineffective (Charbonneau et al. 1986a; Plaa et al. 1982; Traiger and Plaa 1973). In rats given the minimal effective dose of acetone (196 mg/kg) twice daily for 3 days (total dose 1,177 mg/kg), carbon tetrachloride-induced liver toxicity was further enhanced over that of a single dose of 196 mg/kg acetone (Plaa et al. 1982). However, the repetitive dose of acetone (6x196 mg/kg = 1,177 mg/kg) potentiated the liver toxicity of carbon tetrachloride to a lesser extent than a single dose of 1,177 mg/kg. Administration of the noneffective dose (78 mg/kg) twice a day for 3 days (total dose 468 mg/kg) did not affect the liver toxicity of carbon tetrachloride, even though the cumulative dose of 468 mg/kg, if given as a single dose, would have been high enough to cause significant potentiation. When a dose of acetone of 1.5 mL/kg (1,177 mg/kg) was given once, divided into 6 doses of 0.25 mL/kg (196 mg/kg) over 3 days (cumulative dose 1,177 mg/kg) or into 12 doses of 0.125 mL/kg (98 mg/kg) over 3 days (cumulative dose 1,177 mg/kg), or infused intravenously over 3 days, before challenge with carbon tetrachloride, the most severe potentiation occurred with the single dose, followed by 6 divided doses, and, then by 12 divided doses. The intravenous infusion did not enhance the toxicity of carbon tetrachloride. The maximum blood levels calculated from pharmacokinetic parameters for the different acetone treatment regimens showed a direct relationship with the degree of potentiation. The results indicate that threshold blood, and hence liver, concentrations must be exceeded before potentiation occurs. Acetone pretreatment also prolonged the liver toxicity induced by carbon tetrachloride and decreased the recovery time (Charbonneau et al. 1985). With carbon tetrachloride alone, the severity of liver toxicity increased temporally in rats sacrificed 24 and 48 hours after dosing, but liver toxicity was no longer observed at 96 hours. Following pretreatment with acetone, the liver toxicity induced by carbon tetrachloride was enhanced and increased in severity at all sacrifice times, even at 96 hours. Gavage pretreatment of rats with 1,452 mg/kg/day acetone in corn oil twice weekly for ≤12 weeks, followed by carbon tetrachloride

challenge, resulted in decreased body weight gain and 35% mortality, compared with no effect on body weight gain and 5% mortality in rats given corn oil and challenged with carbon tetrachloride (Charbonneau et al. 1986c). The acetone plus carbon tetrachloride treated rats also had statistically significantly decreased relative liver weights and statistically significantly increased kidney weights at all four sacrifice times, compared with corn oil plus carbon tetrachloride rats. Bilirubin concentrations and collagen content were also enhanced. Histological examination revealed fully developed cirrhosis in the acetone plus carbon tetrachloride rats, compared with less severe cirrhosis in the corn oil plus carbon tetrachloride rats. Renal toxicity was also enhanced, as evidenced by statistically significantly elevated blood urea nitrogen levels in the acetone pretreated rats, compared with corn oil pretreated rats. It should be noted that acetone displays a greater degree of potentiation when it is administered in corn oil than in water (Charbonneau et al. 1986a, 1991), and it appears that corn oil alone can be toxic to the liver (Charbonneau et al. 1991).

Inhalation exposure of rats to acetone vapors also displays a threshold effect (Charbonneau et al. 1986a). In rats exposed to 1,000, 2,500, 5,000, 10,000, or 15,000 ppm acetone for four hours, and challenged 18 hours later with carbon tetrachloride, the liver toxicity of carbon tetrachloride was enhanced in a concentration-related manner at $\geq$2,500 ppm acetone. The noneffective concentration was 1,000 ppm. No cumulative effect of repetitive inhalation exposure to acetone on the carbon tetrachloride-induced liver toxicity was found, but maximum blood levels of acetone correlated with the degree of potentiation.

When rats were challenged with a mixture of trichloroethylene and carbon tetrachloride, the minimal effective dose of acetone required to enhance the liver toxicity of carbon tetrachloride decreased at least five-fold, indicating that mixtures of haloalkanes can cause severe liver injury, and prior exposure to acetone can markedly affect the response produced by the mixtures (Charbonneau et al. 1988).

The mechanism of acetone potentiation of carbon tetrachloride-induced liver toxicity involves the induction of mixed function oxidase microsomal enzymes. In microsomes prepared from rats treated by gavage with acetone at 2.5 mL/kg (1,961 mg/kg) and incubated with ^{14}C-carbon tetrachloride, covalent binding of radioactivity to microsomal protein increased 3-4 times that of control microsomes (Sipes et al. 1973). The time course followed the increased activity of N-nitrosodimethylamine N-demethylase, indicating enzyme induction. Furthermore, aminothiazole, an inhibitor of cytochrome P-450 and mixed function oxidase induction, reduced the potentiation by acetone of carbon

tetrachloride-induced liver toxicity (Traiger and Plaa 1973). More recent studies have indicated that the effects of acetone on the toxicity of carbon tetrachloride are caused by induction of cytochrome P-450 forms belonging to at least two gene subfamilies, i.e., P-45011B and P-45011E (Johansson et al. 1988; Kobusch et al. 1989). Complementary DNA and protein sequencing analyses have shown that these P-450 gene subfamilies are similar in rats and humans (Song et al. 1986). Acetone treatment caused a nine-fold increase in cytochrome P-450IIEl accompanied by a similar increase in the rate of nicotinamide adenine dinucleotide phosphate (NADPH)-dependent metabolism of carbon tetrachloride (Johansson et al. 1988). Acetone treatment also increased the amount of messenger ribonucleic acid (mRNA) and apoprotein of P-450IIBl l0- to 30-fold, suggesting regulation of cytochrome P-450IIBl at the transcriptional level. mRNA coding for P-45OIIEl was increased by a combination of fasting and acetone treatment, but not by treatment with acetone alone. The results suggested an enhanced rate of P-45OIIEl gene transcription, P-450IIEl mRNA stabilization, or other posttranscriptional mechanisms. The exact mechanism by which acetone increases the P-450 subfamilies is a subject of recent and on-going investigations. The findings that pretreatment of rats or rabbits with acetone results in increases in cytochrome P-45OIIEl and associated enzyme activities, but has no effect on the level of P-450IIEl mRNA suggests that regulation of acetone-induced P-450IIEl occurs at the posttranscriptional level (Hong et al. 1987; Johansson et al. 1988; Porter et al. 1989; Ronis and Ingelman-Sundberg 1989; Ronis et al. 1991; Song et al. 1986; Song et al. 1989). In microsomal and ribosomal preparations from rats administered acetone intraperitoneally, the polyribosomal distribution of cytochrome P-45OIIEl mRNA shifted, compared with controls, suggesting that the induction of P-45OIIEl by acetone involved enhanced translation efficiency through increased loading of ribosomes of P-45OIIEl mRNA (Kim et al. 1990). However, in another study, incorporation of ^{3}H-leucine into P-45OIIEl in microsomes from rats treated with acetone was lower than that in control microsomes, but the rate of translation of the P-450IIEl mRNA was about the same in both sets of microsomes, indicating that P-45OIIEl is not induced by an increase in the rate of translation of its mRNA (Song et al. 1989). Furthermore, incorporation of $NaH^{14}C0^{3}$ was three-fold less in acetone induced P-450IIEl protein than in controls. The rate of disappearance of radiolabel from P-45OIIEl in controls was biphasic, with half-lives of 7 and 37 hours for the fast and slow phase, respectively. However, in acetone-treated rats, the fast phase was absent, with a monophasic half-life of 37 hours. These results demonstrated that the induction of P-450IIEl by acetone is due primarily to protein stabilization. In microsomes and lysosomes from rats treated with acetone, cytochromes P-45OIIEl and P-450IIBl increased, but the increase was greater in microsomes (Ronis and Ingelman-Sundberg 1989; Ronis et al. 1991). Quantification of the proteins in lysosomes indicated that P-450IIEl and P-450IIBl are

degraded via an autophagosomal/autolysosomal pathway. The authors speculated that P-450IIEl is catalytically inactivated in microsomes prior to degradation in lysosomes and that acetone may interfere with the inactivation. Thus, the induction P-450IIBl appears to occur at the transcriptional level, while the induction of P-450IIEl by acetone appears to occur through stabilization of the apoprotein.

In contrast, pretreatment of mice with carbon tetrachloride enhanced the toxicity of acetone. In mice intraperitoneally pretreated with olive oil, the oral LD_{50} of acetone was 5,250 mg/kg, while in mice pretreated with a 20% solution of carbon tetrachloride in olive oil reduced the LD_{50} of acetone to 4,260 mg/kg (Tanii et al. 1986). The dose of carbon tetrachloride alone did not result in any death. The authors suggested that carbon tetrachloride inactivated the microsomal monooxygenase system, thereby inhibiting the inactivation of acetone.

Acetone also potentiates the hepato- and nephrotoxicity of chloroform. Pretreating rats with 15 mmol/kg (871 mg/kg) acetone in corn oil by gavage 18 hours prior to a challenge dose of 0.5 mL/kg chloroform in corn oil statistically significantly increased the relative kidney weight, inhibited lactate stimulated accumulation of p-aminohippurate and the accumulation of tetraethylammonium ion in kidney slices, and resulted in vacuolar degeneration in the tubular epithelium, but not necrosis, compared with corn oil controls (Hewitt et al. 1980). No effects on these parameters were observed with acetone alone or in rats pretreated with corn oil and challenged with chloroform. Acetone pretreatment also statistically significantly increased the plasma activities of alanine aminotransferase (32-fold) and ornithine carbamoyltransferase (134-fold), compared with corn oil pretreated controls challenged with chloroform, and caused balloon cells with pyknotic nuclei in the centrilobular region of the liver. Acetone alone and chloroform alone did not cause liver lesions. In rats treated by gavage with acetone in corn oil at 58, 290, 436, 581, 726, or 871 mg/kg and challenged with 0.5 mL/kg chloroform in corn oil, acetone showed a dose-dependent decrease in p-aminohippurate uptake and an increase in plasma creatinine levels, with maximum effects seen at doses between 290 and 581 mg/kg acetone (Brown and Hewitt 1984). Renal necrosis, hyaline bodies, and/or tubular casts were seen in 3/6 rats at 58 mg/kg acetone and in 4/6-5/6 rats at higher doses. Acetone pretreatment also statistically significantly increased plasma activities of alanine aminotransferase at $\geq$290 mg/kg. Balloon cells and necrosis were observed in 216 rats pretreated with 58 mg/kg and in most of the rats pretreated with $\geq$290 mg/kg. The effects of acetone pretreatment and chloroform challenge were greater than the effects of corn oil pretreatment and chloroform challenge. Pretreating rats by gavage with 0.5 mL/kg

acetone (871 mg/kg) in corn oil prior to a challenge dose of chloroform (0.5 mL/kg) in corn oil statistically significantly increased the plasma activities of alanine aminotransferase and ornithine carbamoyltransferase above that seen in rats pretreated with corn oil and challenged with chloroform, and the potentiation was maximal at 18 hours (Hewitt et al. 1987). Microsomes from the acetone treated rats showed increased activities of ethoxycoumarin O-deethylase and NADPH-dependent cytochrome c reductase and statistically significantly increased rates of covalent binding of radioactivity from ^{14}C-chloroform in the reaction medium, compared with control microsomes. Results were similar with microsomes prepared from rats treated by gavage with 2.5 mL/kg (1,961 mg/kg) acetone in corn oil. Acetone enhanced the covalent binding of radioactivity of ^{14}C-chloroform two-fold compared with control microsomes and increased the activity of N-nitrosodimethylamine N-demethylase (Sipes et al. 1973), an activity associated with cytochrome P-450IIEl. Thus, acetone increased the biotransformation of chloroform.

The involvement of cytochrome P-450IIEl was confirmed with microsomes from rats given a gavage dose of acetone (871 mg/kg) in corn oil (Brady et al. 1989). Acetone statistically significantly increased the cytochrome P-450 content, the activity of N-nitrosodimethylamine N-demethylase, and the content of cytochrome P-450IIE1, but not P-450IIB1, compared with control microsomes. Furthermore, no effect was seen on the activity of benzphetamine demethylase, an activity associated with P-45011B 1. The acetone-induced microsomes also showed a three-fold enhancement of P-450IIEl-dependent chloroform metabolism, but the activity required the presence of cytochrome b_5 No increased P-45011B l-dependent metabolism was seen. The involvement of P-450IIEl was further demonstrated by inhibition of the reaction with a monoclonal antibody to P-450IIEl and by alternate substrates for P-450IIE1, such as, pyrazole, benzene, nitrosodimethylamine, and diallyl sulfate.

Acetone also potentiates the toxicity of other halogenated alkanes. In rats injected intraperitoneally with acetone in saline at doses of 581, 1,162, 1,742, or 2,323 mg/kg 48 hours prior to a gavage dose of dichloromethane (0.4 mL/kg), statistically significantly increased blood levels of carboxyhemoglobin were observed at 21,742 mg/kg acetone, compared with controls challenged with dichloromethane (Pankow and Hoffmann 1989). The results indicated that acetone increased the metabolism of dichloromethane to carbon monoxide. Results obtained with fasting rats or rats pretreated with isoniazid, which also induces cytochrome P-450IIE1, produced similar potentiation of dichloromethane-induced carboxyhemoglobinemia, thus implicating induction of cytochrome P-450IIEl as the mechanism whereby acetone increased the metabolism of dichloromethane to carbon monoxide.

While neither bromodichloromethane or dibromochloromethane were hepatotoxic (assessed by relative liver weight and plasma activities of alanine aminotransferase and ornithine carbamoyltransferase) in rats at the sublethal doses used, acetone pretreatment at 871 mg/kg by gavage in water resulted in liver toxicity at lower challenge doses of these compounds compared to the doses that produced toxicity when these compounds were administered without acetone pretreatment (Hewitt et al. 1983). Neither bromodichloromethane or dibromochloromethane alone displayed appreciable toxicity to the kidney (assessed by relative kidney weight, accumulation of p-aminohippurate and tetraethylammonium ion in kidney slices, and blood urea nitrogen). However, pretreatment with acetone resulted in statistically significantly increased kidney weight, inhibition of p-aminohippurate uptake, and increased levels of blood urea nitrogen after challenge with bromodichloromethane. With a challenge dose of dibromochloromethane, only blood urea nitrogen was significantly increased by acetone pretreatment. Acetone pretreatment of rats by gavage at doses of 196 and 392 mg/kg, prior to challenge with 1,1,2-trichloroethane, potentiated 1,1,2-trichloroethane-induced increased activity of plasma alanine aminotransferase (MacDonald et al. 1982a). However, higher pretreatment doses of acetone did not potentiate the toxicity and may have decreased the severity. Pretreatment of rats with acetone (392 mg/kg) followed by a challenge dose of ^{14}C-1,1,2-trichloroethane did not increase covalent binding of radioactivity to microsomal proteins but resulted in a greater decline in the content of reduced glutathione. When ^{14}C-trichloroethane was added *in vitro*, covalent binding of the radiolabel statistically significantly increased in microsomes from rats treated with acetone, compared with control microsomes (MacDonald et al. 1982b). The *in vitro* covalent binding was inhibited 80% by the addition of reduced glutathione. It was suggested that acetone alters the bioactivation and the detoxication of 1,1,2-trichloroethane, but the exact mechanism is unclear.

Acetone also potentiated the hepatotoxicity of chlorinated alkenes. Inhalation exposure of rats to 10,000 ppm acetone vapor for 2 hours prior to or during concomitant inhalation exposure to 2,000 ppm 1,l -dichloroethene resulted in statistically significant increased activity of serum alpha-ketoglutarate transaminase, compared with that induced by l,l-dichloroethene alone (Jaeger et al. 1975). A biphasic pattern of potentiation of the liver toxicity induced by l,l-dichloroethene was observed in rats pretreated orally with acetone at several dose levels (Hewitt and Plaa 1983). At doses of 290 and 581 mg/kg acetone prior to challenge with 1,l-dichloroethene, statistically significantly increased activities of plasma alanine aminotransferase and ornithine carbamoyltransferase were observed, compared with water pretreated rats challenged with 1,l-dichloroethene. At higher pretreatment doses of acetone ($\geq$871 mg/kg), the effect on these parameters diminished and acetone

appeared to have a protective effect. Treatment of rats with l,l-dichloroethene did not result in any evidence of nephrotoxicity, but acetone pretreatment statistically significantly reduced the accumulation of tetraethylammonium ion in kidney slices. The biphasic pattern of potentiation/protection may be related to alterations in the rate and/or pattern of l,l-dichloroethene bioactivation, such as, bioactivation to reactive intermediates or decreased detoxication by decreasing hepatic glutathione levels at the potentiating doses of acetone.

Benzene. Although potentiation of benzene toxicity by acetone has not been specifically tested, microsomes from rats treated with acetone (3,922 mg/kg) for 1 or 2-days produced a ≤9-fold increase in the rate of NADPH-dependent oxidation of benzene and induced cytochrome P-450, in particular cytochrome P-450j (cytochrome P-450IIEl) (Johansson et al. 1988; Johansson and Ingelman-Sundberg 1988). Addition of inhibitors of P-450IIEl inhibited the oxidation of benzene in microsomes from acetone treated rats, providing further evidence that this form of cytochrome P-450 is involved. In addition, antibodies to rabbit cytochrome P-450LMeb (IIEl) and rat cytochrome P-450j (IIEl) inhibited the oxidation of benzene by 80-100% in microsomes prepared from rabbits and rats treated with acetone. In hepatocytes from rabbits given acetone (863 mg/kg/day) in drinking water for 7 days, immunoblot analysis identified three distinct cytochromes: P-450IIE1, P-450IA1, and P-450IA2 (Schnier et al. 1989). In bone marrow cells from the treated rabbits, P450IIEl and P-450IAl were identified. Quantitative analysis revealed that acetone treatment resulted in a 7.3-fold induction of P-450IIEl in liver and a 12.9-fold induction of P-450IIEl in bone marrow cells. Acetone slightly decreased the concentration of cytochrome P-450 reductase in bone marrow, and increased the ratio of P-450IIEl to reductase by 16.4 times and the ratio of P-450IAl to reductase by 2 times. Hepatic microsomes from acetone-treated rabbits were 4.8 times more active than control microsomes in benzene hydroxylation, an activity of P-450IIEl. Acetone-induced marrow microsomes were 9.4 times more active in benzene hydroxylation. Thus, the stimulation of benzene metabolism by acetone occurs by a mechanism similar to that of the stimulation of carbon tetrachloride metabolism by acetone. The results suggest that acetone may potentiate the toxicity of benzene, since bioactivation is required for the expression of hematotoxicity of benzene (Sammet al. 1979; Snyder et al. 1975). It should be noted that commercial acetone contains 30 ppm benzene (Union Carbide 1992).

Dichlorobenzene. Inhalation exposure of rats to acetone vapors at 4,785, 10,670, or 14,790 ppm for 4 hours increased the cytochrome P-450 contents and the activity of glutathione-S-transferase, with the greatest increases occurring at the 4,785 ppm level (Brondeau et al. 1989). When the rats were

challenged 18 hours later by inhalation exposure to 1,2-dichlorobenzene, the level of P-450 and the activity of glutathione-S-transferase was no different from that seen with acetone alone. However, acetone preexposure potentiated the liver toxicity of 1,2-dichlorobenzene at the lowest exposure, reduced it at 10,670 ppm, and suppressed it at 14,790 ppm. In mice exposed to 6,747, 8,910, or 14,345 ppm acetone for 4 hours, followed by a challenge by l,2-dichlorobenzene, acetone preexposure caused an interactive glucose-6-phosphatase response in the mediolobular area of the liver. It was suggested that, at low concentrations, acetone induces the microsomal enzymes that convert 1,2-dichlorobenzene to toxic intermediates. However, since the glutathione-S-transferase activity did not increase in rats preexposed to acetone and challenged with 1,2-dichlorobenzene, the diminished liver toxicity induced by 1,2-dichlorobenzene after preexposure to the higher concentrations cannot be explained by detoxification via enhanced glutathione conjugation. Instead, two microsomal enzymes may be involved, in which, at low concentrations of acetone, one (activating) enzyme is induced, but at higher concentrations concomitant induction of the second enzyme system could result in protection.

Ethanol. Acetone potentiated the central nervous system toxicity of ethanol in mice (Cunningham et al. 1989). Mice were pretreated with an intraperitoneal injection of acetone in corn oil at 581, 1,162, or 2,323 mg/kg, and 30 minutes later injected with 4,000 mg/kg ethanol. At 1,162 and 2,323 mg/kg, acetone statistically significantly prolonged the duration of the loss of righting reflex induced by ethanol. In mice given 2,323 mg/kg acetone prior to 2,000 mg/kg ethanol, the blood level of ethanol was statistically significantly higher at all time intervals measured, and acetone pretreatment significantly decreased the mean elimination rate of ethanol.

In vitro, acetone inhibited the activity of liver alcohol dehydrogenase, a reaction responsible for 90% of ethanol elimination. It was suggested that acetone produced a prolongation of the central nervous system toxicity of ethanol by reducing its elimination.

Other Ketones. The neurological and reproductive effects of coexposure to acetone and 2,5-hexanedione has been studied in animals. In rats exposed to 0.5% 2,5-hexanedione, 0.5% acetone (650 mg/kg/day), or to a combination of 0.5% 2,5-hexanedione and 0.5% acetone in drinking water for 6 weeks, peripheral motor nerve conduction velocity was measured weekly from the third week of dosing (Ladefoged et al. 1989). Acetone alone reduced the nerve conduction velocity compared with controls only at 6 weeks, while 2,5-hexanedione alone significantly reduced it from the third week on. The combination treatment resulted in a statistically significantly greater reduction than seen with

2,5-hexanedione alone on the fourth and sixth week. Acetone alone had no effect on balance time in the rotorod test, but balance time was statistically significantly reduced from the second week with the combination treatment, and the reduction was greater than that with 2,5-hexanedione alone from the fourth week on. In a similar dosing regimen for 7 weeks, coexposure to 2,5-hexanedione and acetone statistically significantly inhibited acquisition, but not performance of spatial learning, (assessed in the radial arm maze) above that seen with 2,5-hexanedione alone (Lam et al. 1991). Brain weights of rats exposed to 2,5-hexanedione alone or to the combination were significantly reduced, with greater reduction in the coexposed group. Both treatments reduced synaptosomal 5-hydroxytryptamine uptake rate, but the combination treatment did not reduce the uptake below that seen with 2,5-hexanedione alone. In a companion report of these treatment groups, there was no significant difference on the number and size of neurons in the cerebral cortex between rats treated with 2,5-hexanedione alone or rats coexposed to 2,5-hexanedione and acetone (Strange et al. 1991).

Acetone alone had no effect on indices of fertility in male rats but potentiated the reproductive toxicity of 2,5-hexanedione when coadministered, compared with that seen with 2,5-hexanedione alone (Larsen et al. 1991). The rats were exposed to drinking water containing 0.13%, 0.25%, or 0.5% 2,5-hexanedione or in combination with 0.5% acetone for 6 weeks. Fertility was assessed by mating the exposed males with nonexposed females. 2,5-Hexanedione alone or the combination had no effects on the number of matings. 2,5-Hexanedione alone at 0.5% statistically significantly decreased the number of pregnancies, the number of fetuses, and the testicular weight. The combination treatments further reduced all indices, and at 0.5% 2,5-hexanedione plus 0.5% acetone, complete infertility occurred. Morphological assessment of the testes revealed mild to moderate vacuolization, chromatin margination, epithelial disruption, multinucleated giant cells, and/or atrophy in rats exposed to 2,5-hexanedione alone after 6 weeks of treatment, and the combination increased the severity of these lesions. When assessed 10 weeks after the end of treatment, the lesions were still present.

The mechanism by which acetone potentiates or adds to the toxicity of 2,5-hexanedione in rats is not known, but coexposure of rabbits to 2,5-hexanedione and acetone altered the pharmacokinetic parameters of 2,5-hexanedione (Ladefoged and Perbellini 1986). The combined treatment decreased the body clearance of 2,5-hexanedione, compared to the clearance of 2,5-hexanedione alone.

In a neurobehavioral study in volunteers, 11 men and 11 women exposed to 237 ppm acetone, 12 men and 13 women exposed to 200 ppm 2-butanone (methyl ethyl ketone), and 8 men and 13 women

exposed simultaneously to acetone (125 ppm) and 2-butanone (100 ppm) for 4 hours were subjected to psychomotor tests (choice reaction time, visual vigilance, dual task, memory scanning), sensimotor tests (postural sway), and psychological tests (profile of mood states) (Dick et al. 1989). Acetone exposure alone produced small, but statistically significant changes in performances from controls in two measures of auditory tone discrimination (increase response time and increase false alarm) and hostility in men only. Neither 2-butanone alone or the combination of acetone and 2-butanone produced any statistically significant changes. Furthermore, no interactions between acetone and 2-butanone on the uptake or elimination of acetone or 2-butanone were found in the same human subjects (Brown et al. 1987). From this limited information, it appears that acetone and 2-butanone do not interact to produce neurological effects.

Styrene. Although no studies were located regarding interactions between acetone and styrene in the expression of toxic effects in animals, several studies have reported that coexposure to acetone and styrene produce different changes in the content or activity of biotransformation enzymes in the liver and lungs, compared with the changes seen with styrene alone (Elovaara et al. 1990, 1991; Vainio and Zitting 1978). However, in humans subjects exposed for 2 hours to 293 mg/m^3 styrene alone or to a mixture of 301 mg/m^3 styrene and 1,240 mg/m^3 (517 ppm) acetone, there was no indication that acetone alters the uptake, distribution, metabolism, or elimination of styrene (Wigaeus et al. 1984).

Nitrusamines. Acetone potentiated the hepatotoxicity of N-nitrosodimethylamine in rats pretreated by gavage with 2.5 mL/kg (1,961 mg/kg) acetone in water 24 hours prior to a challenge intraperitoneal dose of 75 mg/kg N-nitrosodimethylamine (Lorr et al. 1984). The acetone pretreatment doubled the plasma activity of alanine aminotransferase ($p<0.005$) and increased the extent and severity of liver necrosis and hemorrhage, compared with that seen with N-nitrosodimethylamine alone. Microsomes prepared from rats treated with N-nitrosodimethylamine had diminished N-nitrosodimethylamine-N-demethylase activity, compared with microsomes from untreated mice. The results indicate that N-nitrosodimethylamine N-demethylase, an activity associated with cytochrome P-450IIE1, is responsible for the activation of N-nitrosodimethylamine to a toxic intermediate, and that the induction of this enzyme by acetone potentiates the hepatotoxicity. Microsomes from mice given 2,614 mg/kg acetone increased the covalent binding of radioactivity from [^{14}C]-N-nitrosodimethylamine to microsomal DNA, RNA, and protein (Sipes et al. 1978). Microsomes from rats pretreated with acetone had a four-fold increased activity of N-nitrosodimethylamine demethylase and a six-fold increase in DNA methylation compared with control microsomes (Hong and Yang 1985). Several

studies have shown that acetone given to rats or mice enhances the microsomal activity of N-nitrosodimethylamine N-demethylase in a dose-related manner (Miller and Yang 1984; Patten et al. 1986; Sipes et al. 1973, 1978; Tu et al. 1983; Yoo et al. 1990), and this activity is associated with cytochrome P-450 (Miller and Yang 1984; Tu et al. 1983; Yoo et al. 1990), in particular cytochrome P-45OIIEl (Patten et al. 1986; Yoo et al. 1990). Acetone pretreatment of rats also enhanced the denitrosation of N-nitroso-dimethylamine in microsomes, and antibodies against cytochrome P-450IIEl inhibited this activity (Yoo et al. 1990). Similar results were obtained with N-nitrosodiethylamine deethylation and denitrosation. The rates of both types of reactions depended upon the concentration of the nitrosamine in the reaction mixture, leading to the conclusion that P-45OIIEl has a role in the metabolism of low concentrations of these nitrosamines, and that this form of the enzyme is important in the carcinogen activation.

In Ames assays, addition of acetone to the S-9 mix inhibited the mutagenicities of N-nitrosodimethylamine, N-nitrosodiethylamine, 6 derivative of N-nitrosopropylamine, and N-nitroso-2,6-dimethylmorpholine in *S. typhimurium* Tal00 at a concentration of ≤5.2 mg/0.l mL (52,000 mg/L) nitrosamines (Mori et al. 1985). Acetone also inhibited the metabolism of N-nitrosodimethylamine, N-nitrosomethyl (2-hydroxypropyl)amine, and N-nitrosomethyl (2-0xopropyl)amine *in vitro.* In contrast, another study found that the S-9 mix prepared from mice treated with acetone strongly enhanced the mutagenicity of N-nitrosodimethylamine in the Ames assay in *S. typhimurium* TA92, which was more sensitive to N-nitrosodimethylamine than TAl00 (Glatt et al. 1981). This assay used concentrations of the nitrosamine at ≤20 mM (1,491 mg/L). However, acetone did not enhance the mutagenicity in the host-mediated assay. The authors explained that *in vitro,* the activity of the dilute metabolizing system is limiting for the activity of N-nitrosodimethylamine, such that induction increases mutagenicity, whereas *in vivo,* N-nitrosodimethylamine is completely metabolized in both induced and noninduced animals. The reason for the different effects of acetone on the mutagenicity of nitrosamines in the studies by Mori et al. (1985) and Glatt et al. (1981) could be related to differences in the assay system (e.g., acetone added to medium versus acetone-induced S-9), to the difference in concentration of the nitrosamines, or to the different strains of *S. typhimurium.* Microsomes from rats pretreated with acetone increased the activity of N-nitrosodimethylamine demethylase and increased the mutagenicity of N-nitrosodimethylamine in Chinese hamster lung V79 cell cultures at low substrate concentrations (0.1 and 4 mM or 5.8 and 232 mg/L) compared with untreated microsomes (Yoo and Yang 1985). However, a slight decrease in mutagenic activity was found at a N-nitrosodimethylamine concentration

of 200 mM (11,616 mg/L). Acetone induced microsomes also enhanced the mutagenic activity of N-nitrosomethylethylamine, N-nitrosodiethylamine, and N-nitrosomethylbutylamine, but not N-nitrosomethylbenzylamine or N-nitrosomethylaniline. The findings that lower concentrations enhanced the mutagenicity of N-nitrosodimethylamine (Glatt et al. 1981; Yoo and Yang 1985) are consistent with the conclusions of Yoo et al. (1990) that cytochrome P-450IIEl is important in the activation of the carcinogen at low concentrations.

Acetonitrile. Acetone also potentiates the toxicity of acetonitrile. When rats were given a 1:l mixture of acetone plus acetonitrile by gavage, the acute LD_{50} was 3-4 times lower than the predicted LD_{50} for additive toxicity (Freeman and Hayes 1985). The LD_{50} values of these chemicals alone were 5,800 mg/kg for acetone and 4,050 mg/kg for acetonitrile, while the LD_{50} for the mixture was 1,160 mg/kg, compared with the predicted value of 4,770 mg/kg. However, deaths occurred later with the mixture than with either acetone or acetonitrile alone. Blood cyanide (a toxic metabolite of acetonitrile) levels were higher, but peaked at a later time, in the rats given the mixture than in those given acetonitrile alone. Administration of a second dose of acetone 30 hours after administration of the mixture protected the rats from lethality to a degree similar to that seen with a dose of sodium thiosulfate (an antidote used for cyanide poisoning). It was suggested that, initially, acetone competitively inhibits the metabolism of acetonitrile to cyanide but later induces an isoenzyme of cytochrome P-450 that catalyzes the metabolism of acetonitrile to cyanide, hence explaining the greater toxicity of the mixture seen at a later time. To test this hypothesis, the metabolism of acetonitrile by microsomes from rats treated with acetone at the same dose that potentiated the toxicity was compared with that by noninduced microsomes (Freeman and Hayes 1988). The metabolism of acetonitrile required oxygen and NADPH and was inhibited by known inhibitors of cytochrome P-450. Microsomes from acetone pretreated rats increased the V_{max}, while acetone added to the reaction mixture *in vitro* competitively inhibited the conversion of acetonitrile to cyanide. The *in vitro* metabolism of acetonitrile was competitively inhibited by ethanol, which also induces cytochrome P-450IIE1, by dimethyl sulfoxide, which inhibits cytochrome P-450IIEl-dependent metabolism of ethanol, and by aniline, a substrate for P-45011El. Thus, the mechanism for the potentiation of the toxicity of acetonitrile by acetone also appears to involve cytochrome P-450IIEl.

A case report describes a woman who was asymptomatic for 24 hours after ingesting an overdose of acetonitrile and acetone, but subsequently developed cardiovascular collapse, and profound acidosis, and eventually died (Boggild et al. 1990). It was suggested that acetone delayed the onset of

symptoms by initially inhibiting the metabolism of acetonitrile to cyanide, which is consistent with the mechanism proposed by Freeman and Hayes (1988).

Acetaminophen. Acetone has been reported to increase the hepatotoxicity of acetaminophen *in vitro* (Moldeus and Gergely 1980) and *in vivo* (Jeffery et al. 1991). The addition of acetone to phenobarbital-induced rat liver hepatocytes caused a three-fold increase in acetaminophen-glutathione conjugation due to enhanced cytochrome P-450-dependent activation of acetaminophen to a toxic metabolite (Moldeus and Gergely 1980). The addition of acetone to the reaction system also caused loss of hepatocyte viability, which was not seen when acetone or acetaminophen were excluded from the system. According to the suggested mechanism, acetone enhanced a cytochrome P-450-dependent activation of acetaminophen to a metabolite that conjugates with glutathione, thereby depleting hepatic glutathione stores, leading to accumulation of the reactive metabolite. In contrast, pretreatment of rats with 813 or 1,975 mg/kg acetone 18 hours and 1 hour prior to administration of acetaminophen resulted in an increased blood half-life of acetaminophen, a decreased rate constant for acetaminophen mercapturate formation, decreased acetaminophen sulfate formation, and decreased renal elimination of acetaminophen (Price and Jollow 1983). Acetone also decreased the incidence and severity of liver necrosis induced by acetaminophen. The authors suggested that acetone decreased the formation of an acetaminophen reactive metabolite. However, in mice pretreated orally with acetone at 1,900 mg/kg/day for 10 days and then given 600 mg/kg acetaminophen intraperitoneally 6 hours before sacrifice, a greater portion of the liver lobules with necrosis and hemorrhage was observed than when acetaminophen was administered alone (Jeffery et al. 1991). Acetone pretreatment followed by saline injection resulted in no hepatic lesions. When dimethylsulfoxide (DMSO), an inhibitor of cytochrome P-450IIE1, was incubated with microsomes prepared from the acetone-pretreated, acetaminophen-treated mice, a 91% inhibition of acetaminophen-glutathione conjugation was found compared to when DMSO was excluded from the incubation mixture. Presumably, the inhibition of glutathione conjugation by DMSO was due to inhibition of cytochrome P-45011El to form the active metabolite of acetaminophen. Activation of acetaminophen to a reactive metabolite, N-acetyl-*p*-benzoquinone imine, which can bind to tissue macromolecules leading to necrosis at high doses of acetaminophen, is known to be dependent on cytochrome P-450IIEl (Morgan et al. 1983; Raucy et al. 1989). N-acetyl-*p*-benzoquinone imine can also be detoxified via conjugation with glutathione. The addition of acetone to the reaction system enhances the formation of the glutathione conjugate in rat liver microsomes (Liu et al. 1991). These results support a mechanism whereby acetone enhances the cytochrome P-450IIEl-dependent conversion of acetaminophen to N-acetyl-*p*-benzoquinone imine,

which in turn conjugates with glutathione to deactivate it. Thus, acetone could decrease the toxicity of acetaminophen. However, if the dose of acetaminophen is high, leading to more N-acetyl-*p*-benzoquinone imine than can be handled by glutathione detoxification, glutathione is depleted and N-acetyl-*p*-benzoquinone imine accumulates. Thus, the induction of cytochrome P-45OIIEl by acetone to produce enough N-acetyl-*p*-benzoquinone imine from acetaminophen to deplete glutathione would result in an enhancement of acetaminophen-induced toxicity.

Miscellaneous Chemicals. 9,10-Dimethyl-1,2-benzanthracene (DMBA) in acetone was more effective as a carcinogen than DMBA in mineral oil when applied to the tongues of hamsters (Marefat and Shklar 1977). A dose of 581 mg/kg acetone prior to administration of N-(3,5-dichlorophenyl)succinimide (NDPS), a fungicide, enhanced the NDPS-induced increase in blood urea nitrogen and kidney weight, but had no effect on NDPS-induced changes in urine volume or content, organic ion uptake by kidney slices, or renal pathology (Lo et al. 1987). Lower doses of acetone were ineffective. Since NDPS requires bioactivation by cytochrome P-450-dependent microsomal enzymes in the liver before renal toxicity occurs, it appears that acetone potentiated the renal toxicity of NDPS by inducing a cytochrome P-450 capable of the bioactivation. Pretreatment of rats with acetone prior to administration of thiobenzamide enhanced the degree of liver necrosis and serum activity of alanine aminotransferase, while coadministration of acetone and thiobenzamide reduced the extent of liver damage (Chieli et al. 1990). In addition, liver microsomes from acetone treated rats statistically significantly increased the rate of thiobenzamide-S-oxidation, which was dependent on a cytochrome P-450 enzyme. Thiobenzamide competitively inhibited acetone monooxygenase activity, which is highly specific for P-45011El. The results indicated that pretreatment of rats with acetone induces P-450IIE1, leading to enhanced bioactivation of thiobenzamide to a reactive metabolite and enhanced thiobenzamide-induced liver damage. However, when acetone and thiobenzamide were administered together, competition for the enzyme may have led to less bioactivation of thiobenzamide, thereby affording the protective effect of acetone. Acetone appears to afford protection against other toxic effects of other chemicals. Pretreatment of rats with acetone produced complete protection against clonic tonic convulsions induced by isonicotinic acid and electroshock-induced convulsion (Kohli et al. 1967). Since the protective action of acetone was nonspecific, a biochemical mechanism did not seem likely.

Acetone also increased the toxicity of oxygen (Tindberg and Ingelman-Sundberg 1989) and chromate (Cr[VI]) (Mikalsen et al. 1991). Pretreatment of rats with acetone prior to oxygen exposure

potentiated the NADPH-dependent microsomal lipid peroxidation in the liver and lung and decreased the survival of the rats (Tindberg and Ingelman-Sundberg 1989). Oxygen also induced cytochrome P-450IIE1, indicating a role for cytochrome P-450IIEl in oxygen-mediated tissue toxicity. Coexposure of rats to acetone and sodium chromate (Cr[VI]) resulted in some macroscopic alterations in the liver (not otherwise described), whereas no liver toxicity was noted with chromate or acetone alone (Mikalsen et al. 1991). Cytochrome P-450IIEl exhibited high chromate reductase activity, and biochemical studies indicated that acetone caused the induction of microsomal Cr(V1) metabolism.

While the interactions discussed above involve the potentiation of the toxicity of other chemicals by acetone, acetone has been found to antagonize the toxicity of semicarbizide (Jenney and Pfeiffer 1958). In mice injected intraperitoneally with 168 mg/kg semicarbizide, 93% had convulsions and 91% died. Pretreatment with 4,000 mg/kg acetone orally reduced the percentage of the semicarbizide-induced convulsions and mortality to 0%. A dose of 1,800 mg/kg acetone reduced the percentage of mice convulsing to 31%, delayed the onset of convulsions by 286%, reduced the percentage that exhibited unmodified seizure from 98% to 40%, reduced the mortality to 12.5%, and delayed the time to death by 125%. The authors attributed the protective effect of acetone to the presence of the keto group.

2.7 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to acetone than will most persons exposed to the same level of acetone in the environment. Reasons include genetic make-up, developmental stage, age, health and nutritional status (including dietary habits that may increase susceptibility, such as inconsistent diets or nutritional deficiencies), and substance exposure history (including smoking). These parameters result in decreased function of the detoxification and excretory processes (mainly hepatic, renal, and respiratory) or the preexisting compromised function of target organs (including effects or clearance rates and any resulting end-product metabolites). For these reasons we expect the elderly with declining organ function and the youngest of the population with immature and developing organs will generally be more vulnerable to toxic substances than healthy adults. Populations who are at greater risk due to their unusually high exposure are discussed in Section 5.6, “Populations With Potentially High Exposure.”

Several lines of evidence from studies in animals indicate that sex differences exist in the susceptibility to effects caused by acetone. Male rats were more susceptible than female rats to acetone’s

hematological, hepatic, and renal effects, and effects on reproductive organs (American Biogenics Corp 1986; NTP 1991). While results in animals cannot always be extrapolated to humans, it is possible that men may be more susceptible than women to the hematological, hepatic, renal, and reproductive effects of acetone. Furthermore, acetone may exacerbate preexisting hematological, liver, kidney, or reproductive disorders in humans.

In a lethality study among newborn rats, 14-day-old rats, and adult rats, susceptibility to the lethal effects of acetone generally decreased with increasing maturity (Kimura et al. 1971). Humans may have the same order of susceptibility.

Pregnant rats exposed to acetone by inhalation during gestation had reduced body weights (NTP 1991), while nonpregnant rats exposed to a higher concentration for a longer duration did not show any effects on body weight (Goldberg et al. 1964). Pregnant rats also had lower plasma and liver levels of acetol, the first intermediate in the overall metabolism of acetone, than virgin rats (Peinado et al. 1986), suggesting differences in the rate of acetone metabolism. It is possible that the condition of pregnancy made these rats more susceptible to body weight changes, and this susceptibility might apply to humans.

The role of acetone in fasting and diabetes is complicated and not well understood (Reichard et al. 1979; 1986). Acetone is produced endogenously and, as demonstrated in humans, more acetone is produced endogenously by fasting, which can result in ketosis (Reichard et al. 1979). This implies that people on diets exposed to exogenous acetone will have a higher body burden of acetone than nondieters exposed to the same amount of exogenous acetone, perhaps making them more susceptible to any possible adverse effects. Acetone exposure of rats resulted in a reduced insulin-stimulated glucose oxidation rate, and the reduction was greater in fasted rats than in fed rats, indicating that the insulin resistance indigenous to fasting may be attributed in part to metabolic influences of acetone (Skutches et al. 1990). This implies that people on diets may have a diminished capacity to utilize glucose, and exposure to acetone may reduce the capacity further.

Diabetics may also be more susceptible to the effects of acetone. Acetone-induced insulin resistance (Skutches et al. 1990) might also result in greater hyperglycemia in diabetics. Patients with diabetic ketoacidosis have higher plasma levels of endogenous acetone (Reichard et al. 1986), and exposure to exogenous acetone may increase the levels further. Similar results were found in rats. Diabetic rats

had higher plasma acetone levels than nondiabetic rats after treatment with the same doses of acetone, due to the higher endogenous level of acetone in the diabetic rats and differences in the metabolism of acetone to isopropyl alcohol (Lewis et al. 1984). Diabetic rats were also less able to oxidize acetone than nondiabetic rats (Price and Rittenberg 1950).

2.8 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to acetone. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to acetone. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

2.8.1 Reducing Peak Absorption Following Exposure

Since acetone is irritating to mucous membranes of the respiratory system and eyes, people exposed occupationally wear protective clothing, goggles, and respirators (Stutz and Janusz 1988). If exposure has occurred and symptoms of narcosis are present, the victim is removed from the contaminated area, clothing is removed and isolated, the skin is washed with soapy water, oxygen is administered, and eyes are thoroughly flushed with water. In the case of ingestion of acetone, activated charcoal is given. Although induction of emesis by administration of syrup of ipecac is sometimes recommended in the case of ketones in general (Stutz and Janusz 1988), this may be contraindicated in the case of acetone ingestion because of the possibility of pulmonary aspiration, which increases for substances with high volatility and low viscosity (Goldfrank et al. 1990). Gastric lavage has been used to treat a patient who ingested acetone (Sakata et al. 1989), but the possibility of aspiration also exists for this method (Goldfrank et al. 1990).

2.8.2 Reducing Body Burden

Following inhalation or oral exposure, acetone is eliminated within about l-3 days in humans (DiVincenzo et al. 1973; Matsushita et al. 1969a, 1969b; Ramu et al. 1978; Sakata et al. 1989). Acetone does not accumulate in any tissue and its metabolites do not appear to be toxic or retained (Wigaeus et al. 1982). To reduce the body burden of acetone, a cathartic, such as magnesium sulfate

in water, is administered (Stutz and Janusz 1988). In a case of isopropyl alcohol poisoning (acetone is a major metabolite of isopropyl alcohol), hemodialysis has been used successfully to enhance elimination of both isopropyl alcohol and acetone (Rosansky 1982).

2.8.3 Interfering with the Mechanism of Action for Toxic Effects

The mechanism of the narcotic effects of acetone is not known, but as a solvent, acetone may interfere with the composition of the membranes, altering their permeability to ions (Adams and Bayliss 1968). Systemically, acetone is moderately toxic to the liver, and produces hematological effects. The mechanism by which acetone produces these effects is unknown. The renal toxicity may be due to the metabolite, formate, which is known to be nephrotoxic (NTP 1991), and is excreted by the kidneys (Hallier et al. 1981). Furthermore, the renal toxicity, which appears to be specific for male rats, may involve $\alpha_{2\mu}$-globulin syndrome, as hyaline droplet formation was associated with the nephropathy observed in male rats in the American Biogenics Corp. (1986) study. Acetone also causes reproductive effects in male rats, and is fetotoxic. Although the exact mechanism for many of the effects of acetone is not known, distribution studies in mice indicate that acetone and metabolites are found in all of the target organs (Wigaeus et al. 1982). Acetone and some of its metabolites were also transferred to rat fetuses after the dams were exposed to acetone (Peinado et al. 1986). The metabolites of acetone are gluconeogenic precursors and most do not appear to be the toxic. Therefore, acetone itself appears to be a toxic agent, and increasing the metabolism of acetone would appear to be the best method for interfering with the mechanism of action. However, acetone induces its own metabolism by inducing cytochrome P-450IIEl (Johansson et al. 1986; Puccini et al. 1990). The first and second steps of the metabolism of acetone are dependent on cytochrome P-45OIIEl (Casazza et al. 1984; Johansson et al. 1986; Koop and Casazza 1985; Puccini et al. 1990). Since ethanol also induces this particular form of P-450IIEl (Johansson et al. 1988; Puccini et al. 1990), the metabolism of acetone might be increased by administering ethanol, although this may competitively slow acetone metabolism, at first, and induce cytochrome P-450IIEl only after a lag of several hours. In healthy men who fasted for 12 hours, the breath acetone levels ranged from 0.96 to 1.7 ppm (Jones 1987). Fasting for 36 hours resulted in average acetone breath levels of 14-66 ppm. In fasting men who ingested 0.25 g/kg of ethanol, the breath acetone levels decreased by 40% after a 12-hour fast and by 18% after a 36-hour fast (Jones 1988). However, since acetone potentiates the toxicity of other chemicals by inducing cytochrome P-45OIIE1, which enhances the metabolism of the chemicals to reactive intermediates (see Section 2.6), further increasing the cytochrome P-450IIEl levels might be

counterproductive in cases of exposure to acetone followed by exposure to the other chemicals. Furthermore, acetone potentiates the central nervous system toxicity of ethanol by reducing the activity of alcohol dehydrogenase, which is responsible for 90% of ethanol elimination (Cunningham et al. 1989).

2.9 ADEQUACY OF THE DATABASE

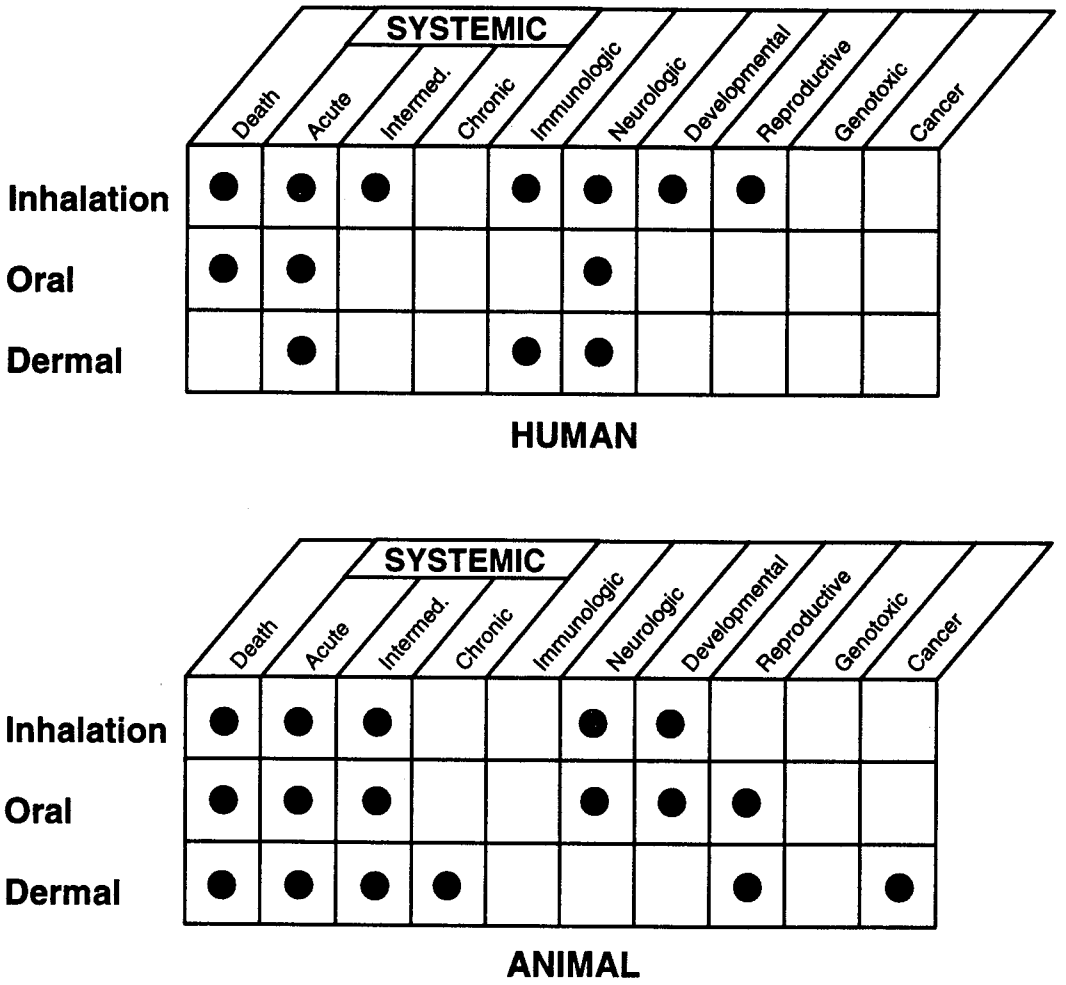
Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of acetone is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of acetone.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

2.9.1 Existing Information on Health Effects of Acetone

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to acetone are summarized in Figure 2-4. The purpose of this figure is to illustrate the existing information concerning the health effects of acetone. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not imply anything about the quality of the study or studies. Gaps in this figure should not be interpreted as “data needs.” A data need, as defined in ATSDR’s Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

FIGURE 2-4. Existing Information on Health Effects of Acetone



● Existing Studies

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As seen from Figure 2-4, data exist for inhalation exposure of humans for death, for systemic effects of acute- and intermediate-duration exposure, and for immunological, neurological, developmental, and reproductive effects. The data for systemic, neurological, immunological, and neurological effects were derived from medical evaluations of workers after a single day exposure, from case reports, and from experimental studies of humans exposed for acute- and intermediate-durations. The systemic effects include respiratory irritation, cardiovascular effects, gastrointestinal effects, and hematological effects, with no indications of hepatic or renal effects. Possible immunological effects consisted of increased white blood cell counts and decreased phagocytic activity of neutrophils. Neurological effects consisted of subjective symptoms, coma, and some behavioral effects. Reproductive and development effects consisted of shortened menstrual periods of women in an experimental study. An epidemiological study of pregnancy outcome among female laboratory workers who handle solvents found no effects, but an unreliable study of pregnancy complications in Russian women factory workers reported reproductive and developmental effects. Information in humans after oral exposure was derived from case reports of intentional or accidental ingestion. Effects included erosions in the buccal cavity, development of diabetes-like symptoms, and coma, and other neurological effects. Effects of dermal/ocular exposure of humans consisted of eye irritation, degenerative changes in the epidermis, and the development of contact dermatitis in a sensitized woman. In addition, patients exposed by inhalation and/or dermally from applications of casts using acetone as a setting agent developed increased pulse rate, vomiting and nausea, and neurological effects, including coma.

For animals exposed by inhalation, data exist for death, systemic effects of acute- and intermediate-duration exposure, and neurological, developmental, and reproductive effects. Systemic effects consisted of respiratory irritation, hepatic effects, renal effects, and body weight changes. Neurological effects consisted of narcosis, coma, and behavioral effects. Fetotoxicity, but no reproductive effects, were found in a developmental study. For oral exposure, data in animals were available for death (acute), systemic effects of acute- and intermediate-duration exposure, and neurological, reproductive, and developmental effects. Systemic effects included respiratory (enzyme induction), hematological, hepatic, and renal effects, and reduced insulin-stimulated glucose oxidation and body weight changes. No cardiovascular, gastrointestinal (other than enzyme induction), musculoskeletal, or dermal/ocular effects were found. Neurological effects consisted of narcotic effects and reduced nerve conduction velocity. Reproductive effects were found in male rats, and fetotoxicity was found in a developmental study. Effects of dermal/ocular exposure consisted of amyloidosis in the heart, liver, kidney, pancreas, and adrenals of mice exposed dermally, eye irritation and cornea1 burns and necrosis, skin irritation,

and cataract development due to dermal exposure. Mice used as acetone solvent controls in skin painting studies did not develop any acetone-related neoplastic or nonneoplastic lesions after comprehensive histological examination. Acetone was negative as a tumor initiator or promotor in skin painting studies.

2.9.2 Identification of Data Needs

Acute-Duration Exposure. Acute inhalation studies in volunteers (Matsushita et al. 1969a, 1969b; Nelson et al. 1943) and on-site medical evaluations of workers on a single work day (Raleigh and McGee et al. 1972; Ross 1973) have found that acetone is irritating to the nose, throat, trachea, lungs, and eyes. Acute experimental studies in humans also found hematological effects, which might also indicate immunological effects (Matsushita et al. 1969a, 1969b) and neurological and neurobehavioral effects (Dick et al. 1989; Haggard et al. 1944; Matsushita et al. 1969a, 1969b; Seeber and Kieswetter 1991; Seeber et al. 1992). Case reports of patients exposed to acetone vapors by inhalation and/or dermally during and after application of hip casts have found cardiovascular effects (increased pulse rates), gastrointestinal effects (vomiting of blood, hemorrhage), and neurological effects (lethargy, headache, listlessness, mystagmus, dizziness, coma, collapse) (Chatterton and Elliot 1946; Fitzpatrick and Claire 1947; Harris and Jackson 1952; Hift and Pate1 1961; Pomerantz 1950; Renshaw and Mitchell 1956; Strong 1984). No indication that the liver and kidney were target organs, based on clinical chemistry parameters, was found in humans exposed under laboratory conditions (DiVincenzo et al. 1973). Information on effects of acute oral exposure in humans was derived from case reports of accidental or intentional ingestion of acetone and indicate that ingestion causes coma (Gamis and Wasserman 1988; Gitelson et al. 1966; Ramu et al. 1978; Sakata et al. 1989), erosions in the buccal cavity, and possible diabetes-like symptoms (Gitelson et al. 1966). Direct application of acetone to the skin of humans resulted in skin irritation and degenerative changes in the epidermis (Lupulescu et al. 1972, 1973; Lupulescu and Birmingham 1975, 1976), and a case of contact dermatitis was reported (Tosti et al. 1988). Acute inhalation studies in animals have provided LC_{50} values for rats (Pozzani et al. 1959) and have identified the respiratory system (irritation) in mice and guinea pigs (Kane et al. 1980; Specht et al. 1939), liver in mice, guinea pigs, and rats (NTP 1988; Specht et al. 1939; Vainio and Zitting 1978), and kidney in guinea pigs (Specht et al. 1939) as possible target organs. The heart and stomach did not appear to be target organs, and the kidney effects in guinea pigs were found at lethal concentrations (Specht et al. 1939). For acute oral exposure in animals, LD_{50} values are available for rats (Freeman and Hayes 1985; Kimura et al. 1971; Pozzani et al. 1959; Smyth et al.

1962), for mice (Tanii et al. 1986), and guinea pigs (Striegel and Carpenter 1961). Oral doses associated with death were also available for rabbits (Walton et al. 1928) and dogs (Albertoni 1884). The systemic effects of acute oral exposure found in animals were enzyme induction in the respiratory system of hamsters (Ueng et al. 1991) and rabbits (Ding and Coon 1990) in the duodenum and jejunum of rats (Carriere et al. 1992), in the bone marrow of rabbits (Schnier et al. 1989), in the liver of rats, mice, rabbits, guinea pigs, and hamsters (see Section 2.2.2.2), and in the kidneys of hamsters (Menacagli et al. 1991; Ueng et al. 1992) and rats (Hong et al. 1987), increased liver weight in mice and bone marrow hypoplasia in rats (Dietz et al. 1991; NTP 1991) kidney effects in rats (Brown and Hewitt 1984), and reduced insulin-stimulated glucose oxidation in adipose tissue in rats (Skutches et al. 1990). Acute dermal exposure of animals produced increased DNA synthesis in the skin, indicative of irritation, in mice (Iversen et al. 1988), and ocular exposure caused cornea1 bums and necrosis in rabbits and guinea pigs (Bolkova and Cejkova 1983; Carpenter and Smyth 1946; Smyth et al. 1962). Acetone does not appear to be very toxic by the dermal route, as attempts to determine LD_{50} values for rabbits and guinea pigs found no deaths at the highest dermally applied concentrations (Roudabush et al. 1965; Smyth et al. 1962). The acute inhalation study of Specht et al. (1939) was conducted at lethal concentrations. An acute inhalation MRL of 26 ppm was derived based on neurobehavioral effects in humans. Most of the acute inhalation and oral studies in animals were designed to study enzyme induction. Comprehensive histological examination was performed in the high dose and control animals in the 14-day drinking water study in rats and mice exposed at several dose levels (Dietz et al. 1991; NTP 1991). An acute oral MRL was not derived because NOAEL and LOAEL values in rats or mice in the acute-duration drinking water studies (Dietz et al. 1991; NTP 1991; Skutches et al. 1990) were close to or fell within the range of LD_{50} values for rats exposed by gavage (Freeman and Hayes 1985; Kimura et al. 1971). Acute-duration inhalation and oral studies conducted at several exposure levels in rats and mice that perform clinical tests and comprehensive histological examination of all dose groups would provide valuable information on dose-response relationships and better characterize target organs, which might serve as bases for the development of MRLs. This information is important because there are populations surrounding hazardous waste sites that might be exposed to acetone for brief periods.

Intermediate-Duration Exposure. The only information located regarding systemic effects in humans after intermediate-duration inhalation exposure was an experimental study in volunteers exposed intermittently to acetone at concentrations $\leq$1,250 ppm for $\leq$7.5 hours and $\leq$5 or 6 weeks in a complex protocol (Stewart 1975). Throat irritation was reported, but pulmonary function testing,

electrocardiography, clinical chemistry and urinalysis determinations for liver and kidney damage, and hematology did not reveal any abnormalities. No studies were located regarding effects in humans after intermediate-duration oral or dermal exposure to acetone. An intermediate-duration inhalation study in rats found no histological or clinical evidence of effects on lungs, hearts, kidneys, or livers (Bruckner and Peterson 1981b), but histological examination was not comprehensive. An intermediate inhalation MRL of 13 ppm was derived based on neurological effects in humans. An intermediateduration drinking water study found increased liver weight in mice and hematological effects (macrocytic anemia), increased liver weight, and kidney effects (nephropathy) in rats (Dietz et al. 1991; NTP 1991). An intermediate-duration gavage study in rats found clinical evidence of liver effects, hematological effects, and nephropathy (American Biogenics Corp. 1986). The renal effects seen in these studies appeared to be specific for male rats and may be related to $\alpha_{2\mu}$-globulin syndrome, since hyaline droplet formation was observed in the American Biogenics Corp. (1986) study, but not the NTP (1991) study. No other systemic target organs were identified by comprehensive histological examination. An intermediate-duration oral MRL of 2 mg/kg/day was derived based on the NOAEL for macrocytic anemia in rats in the 13-week drinking water study (Dietz et al. 1991; NTP 1991). Intermediate-duration dermal exposure of animals resulted in moderate hyperplasia of the epidermis of mice (Iversen et al. 1981), mild erythema and transient weight loss in guinea pigs (Taylor et al. 1993), cataracts in the eyes of guinea pigs (Rengstorff et al. 1972), and amyloidosis in the heart, liver, kidney, skin, pancreas, and adrenals of mice (Barr-Nea and Wolman 1977). Intermediate-duration ocular exposure of rabbits resulted in uveal melanocytic hyperplasia (Pe’er et al. 1992). An intermediate-duration inhalation study in rats and mice that is designed to establish dose-response data for noncancer end points would also provide dose-response relationships and identify target organs useful for MRL derivation and fill this data gap. An intermediate-duration drinking water study in rats conducted at lower exposure levels than those used in the NTP (1991) study might provide information on the threshold for anemia and nephropathy. Furthermore, a study designed to confirm whether the acetone-induced nephrotoxicity is related to $\alpha_{2\mu}$-globulin syndrome would aid in the assessment of the relevance of the renal effects to human health. Examination of workers who handle liquid acetone might provide information on the relevance to humans of the cataract formation and amyloidosis seen in animals. This information is important because there are populations surrounding hazardous waste sites that might be exposed to acetone for similar durations.

Chronic-Duration Exposure and Cancer. A retrospective mortality study of workers exposed to acetone found no significant excess risk of death from any cause (all causes, malignant neoplasm,

circulatory system disease, ischemic heart disease) (Ott et al. 1983a, 1983b). Furthermore, a health evaluation survey of active employees found no evidence of hematological or liver effects (Ott et al. 1983a, 1983c). No studies were located regarding systemic effects in humans after oral or dermal exposure to acetone or in animals after inhalation or oral exposure for chronic durations. The only information regarding systemic effects in animals after chronic-duration exposure to acetone is that female mice used as acetone solvent controls in skin painting studies did not develop any acetone-related nonneoplastic lesions after comprehensive histological examination (Ward et al. 1986) and that chronic dermal of exposure of mice resulted in low incidences of hyperplasia, dermatitis, and hyperkeratosis (DePass et al. 1989). A chronic inhalation MRL of 13 ppm was derived based on meurological effects in humans in an intermediate-duration study. The lack of data regarding systemic effects after oral exposure precludes the derivation of an oral MRL. The conduct of chronic studies by these routes in rats and mice would provide information on the dose-response relationships and identify target organs of chronic inhalation and oral exposure to acetone. This information is important because there are populations surrounding hazardous waste sites that might be exposed to acetone for long periods of time.

A retrospective mortality study of workers exposed to acetone found no significant excess risk of death from any cause, including malignant neoplasm (Ott et al. 1983a, 1983b). No studies were located regarding cancer in humans after oral or dermal exposure to acetone, or in animals after inhalation or oral exposure. Mice used as acetone solvent controls in skin painting studies did not develop any acetone-related neoplastic lesions after comprehensive histological examination (Ward et al. 1986) or skin tumors (DePass et al. 1989; Van Duuren et al. 1978). Acetone was negative as a tumor initiator (Roe et al. 1972) and as a tumor promoter for 7,12-dimethylbenz[a]anthracene (Roe et al. 1972; Van Duuren et al. 1971; Weiss et al. 1986). Following dermal absorption, acetone is probably widely distributed throughout the body, as occurs after pulmonary absorption (Wigaeus et al. 1982). However, it is not possible to predict that acetone would not be carcinogenic after inhalation or oral exposure, because quantitative data regarding dermal absorption were not located. Genotoxicity studies indicate that acetone may be weakly genotoxic (see below). Chronic inhalation and oral studies in rats and mice designed to establish dose-response data for noncancer end points would also provide information on the potential for acetone to cause cancer.

Genotoxicity. No studies were located regarding genotoxicity in humans or animals after inhalation, oral, or dermal exposure. *In vivo* genotoxicity studies were conducted by the intraperitoneal route for

micronuclei formation in Chinese hamsters (Basler 1986) and for cell transformation in fetal cells from pregnant hamsters (Quarles et al. 1979a, 1979b) with negative results. In addition, tests for gene mutation in silk worms by an unspecified route were negative (Kawachi et al. 1980). Numerous studies were conducted *in vitro*. Mostly negative results were obtained in bacterial (De Flora 1981; De Flora et al. 1984; De Marini et al. 1991; Ishidate et al. 1984; Kawachi et al. 1980; Kubinski et al. 1981; McCann et al. 1975; Rossman et al. 1991; Yamauchi 1985) and yeast (Abbandandolo et al. 1980; Albertini 1991) assays and in plant seeds (Gichner and Veleminsky 1987) with or without metabolic activation, but some results were positive in *E. coli* when acetone was in the triplet state (Menck et al. 1986; Rahn et al. 1974) and in yeast for aneuploidy (Zimmermann 1983; Zimmermann et al. 1984, 1985) and for mitotic chromosome malsegregation (Albertini 1991) without metabolic activation. Mostly negative results were obtained in assays for cell transformation, chromosomal aberrations, sister chromatid exchange, colony formation inhibition, and gene mutation in cultured animal cells (Amacher et al. 1980; Chen et al. 1984; DiPaolo et al. 1969; Freeman et al. 1973; Kawachi et al. 1980; Mishra et al. 1978; Pienta 1980; Rhim et al. 1974; Tates and Kriek 1981), and for sister chromatid exchange and unscheduled DNA synthesis in cultured human fibroblasts and skin epithelial cells (Abe and Sasaki 1982; Kawachi et al. 1980; Lake et al. 1978). However, some positive results were obtained for chromosomal aberrations in Chinese hamster fibroblasts (Ishidate et al. 1984) and hamster lung fibroblasts (Kawachi et al. 1980), for inhibition of metabolic cooperation in Chinese hamster cells (Chen et al. 1984), and for chromosome malsegregation in porcine brain tubulin (Albertini et al. 1988). Acetone did not promote the transforming activity initiated by nine known genotoxic and carcinogenic chemicals (Sakai and Sato 1989). The mostly negative results in bacteria and cultured animal cells and the negative results in human fibroblasts and skin epithelial cells indicate that acetone poses little threat for genotoxicity in humans. However, peripheral lymphocytes, fibroblasts, and skin epithelial cells from workers exposed to acetone could be examined for chromosomal aberrations to confirm this hypothesis.

Reproductive Toxicity. Information regarding reproductive effects in humans after inhalation exposure is limited to the report of premature menstrual periods by 3 of 4 women exposed to 1,000 ppm acetone for 7.5 hours (Stewart et al. 1975), the lack of a statistically significant increased incidence of miscarriage in female laboratory workers exposed to a variety of solvents, including acetone (Axelsson et al. 1984) and an unreliable report of pregnancy complications of Russian women factory workers (Nizyaeva 1982). No studies were located regarding reproductive effects in humans after oral or dermal exposure to acetone. Information regarding reproductive effects in animals after

inhalation exposure is also limited. In an inhalation developmental study in rats and mice, no effects were found on the number of implants/litter, percent live pups/litter, or mean percent resorptions/litter (NTP 1988). No studies were located regarding reproductive effects in male animals, histological effects on reproductive organs of male or female animals, or the reproductive outcomes and other indices of reproductive toxicity in animals after inhalation exposure to acetone. Reproductive effects (reduced reproductive index and increased duration of gestation) were found in pregnant mice exposed orally to 3,500 mg/kg/day during gestation (EHRT 1987). In a 6-week drinking water study in male Wistar rats, no effects were found on successful mating to untreated females, number of pregnancies, number of fetuses, testicular weight, seminiferous tubule diameter, and testicular lesions (Larsen et al. 1991). However, male Sprague-Dawley rats had statistically significantly decreased sperm motility, caudal weight and epididymal weight, and increased incidences of abnormal sperm, but no histopathological testicular lesions in a 13-week drinking water study (NTP 1991). Vaginal cytology examinations of the female rats revealed no effects, and similar evaluations of male and female mice revealed no effects. No indication that dermal exposure of female mice results in histopathological effects in reproductive organs was found in an analysis of female SENCAR mice used as acetone controls in a skin painting study of formaldehyde (Ward et al. 1986). An inhalation distribution study in male mice showed that acetone is distributed to the testes and vas deferens (Wigaeus et al. 1982), and the 13-week oral study showed effects on the male rat reproduction organs. Acetone is widely distributed throughout the body regardless of route of exposure and species. Therefore, it is reasonable that male rats (and perhaps male mice) exposed to acetone by inhalation for 13 weeks might have effects on reproductive organs, but the concentration of acetone that would cause such effects cannot be predicted. Therefore, reproductive organ pathology should be examined in the suggested intermediate-duration inhalation study. The reproductive organs of female rats and mice could also be examined in the intermediate-duration inhalation study to confirm the lack of effects in the existing oral study. A multi-generation study conducted by the oral route would clarify whether the reproductive effects observed in male rats in the existing 13-week drinking water study would affect reproductive outcomes and other indices of reproduction. If the proposed intermediate-duration inhalation study showed reproductive organ pathology, a similar multigeneration study by the inhalation route might be warranted.

Developmental Toxicity. Information regarding developmental effects in humans after inhalation exposure is limited to a report that found no statistically significant increased incidence of miscarriage, perinatal death rate, or malformations of offspring in female laboratory workers exposed to a variety of

solvents, including acetone (Axelsson et al. 1984) and an unreliable report of fetal death and reduced birth weight and length of neonates in Russian women factory workers exposed to acetone (Nizyaeva 1982). No studies were located regarding developmental effects in humans after oral or dermal exposure to acetone. Developmental effects have been found in animals after inhalation and oral exposure. Decreased mean fetal body weight occurred at maternally toxic doses in rats exposed by inhalation during gestation (NTP 1988). Significantly increased incidence of late resorption, decreased fetal weight, and significantly increased incidence of reduced sternebral ossification occurred at maternally toxic doses in mice exposed by inhalation during gestation (NTP 1988). In mice exposed orally during gestation, reduced postnatal pup survival and reduced average weight of each live pup/litter on postpartum day 0 occurred at maternally toxic doses (EHRT 1987). Fetuses or pups were not examined for internal malformations or skeletal anomalies. Therefore, a more conventional oral developmental toxicity study in mice would help to determine whether acetone causes fetal malformations or skeletal anomalies. Developmental studies were not conducted in rats by the oral route, but while it is reasonable to expect that fetotoxicity would occur, it is not possible to predict the dose that would cause fetotoxicity in rats. An oral developmental study in rats would provide this information. No studies were located regarding developmental effects in animals after dermal exposure to acetone. Acetone can undergo transplacental transfer (Peinado et al. 1986). While it appears that acetone is absorbed after dermal exposure, quantitative data were not available. If enough acetone is absorbed dermally, then fetotoxicity by this route would also be expected. A dermal developmental study might confirm or refute this possibility. Acetone has been tested in attempts to develop *in vitro* methods for assessing developmental toxicity in postimplantation rat embryos (Kitchin and Ebron 1984; Schmid 1985) and in mouse embryo limb buds (Guntakatta et al. 1984) with inconclusive results. Further *in vitro* studies could be conducted to resolve the inconsistencies, but such studies would be useful only for preliminary screening purposes and would not substitute for developmental studies in animals administered acetone by environmentally relevant routes.

Immunotoxicity. Information regarding immunological effects in humans after exposure to acetone is limited. Significantly increased white blood cell counts, increased eosinophil counts, and decreased phagocytic activity of neutrophils were found in volunteers exposed by inhalation (Matsushita et al. 1969a, 1969b), but a battery of immune function tests has not been performed. A case report of a laboratory technician described the development of acute contact dermatitis from handling acetone 2 years after being treated with squaric acid dibutyl ester in acetone for patchy alopecia areata on her scalp (Tosti et al. 1988). This acetone sensitization is considered a rare complication of sensitizing

therapies for alopecia areata. No studies were located regarding immunological effects in humans after oral exposure or in animals after inhalation, oral, or dermal exposure to acetone. A study performing a battery of immune function tests would clarify whether acetone is an immunotoxicant.

Neurotoxicity. Exposure to acetone by the inhalation and oral routes has resulted in neurological effects, related to the narcotic effects of acetone, in both humans and animals. Acetone can cause coma and other neurological effects in humans after inhalation and/or dermal exposure (Chatter-ton and Elliott 1946; Fitzpatrick and Claire 1947; Harris and Jackson 1952; Hift and Pate1 1961; Pomerantz 1950; Renshaw and Mitchell 1956; Ross 1973; Strong 1944) or oral exposure (Gamis and Wasserman 1988; Ramu et al. 1978; Sakata et al. 1989) if exposure levels or doses are high enough. Neurological effects were commonly experienced by workers and volunteers exposed by inhalation to acetone and include headache, lightheadedness, dizziness, unsteadiness, and confusion (Raleigh and McGee 1972; Ross 1973). Neurobehavioral tests conducted in some of these workers were negative (Raleigh and McGee et al. 1972). Neurological and behavioral effects have also been observed in volunteers exposed by inhalation (Dick et al. 1989; Haggard et al. 1944; Matsushita et al. 1969a, 1969b; Seeber and Kieswetter 1991; Seeber et al. 1992; Stewart et al. 1975). Neurobehavioral effects, indicative of narcosis, have been observed in rats (Bruckner and Peterson 1981a; Garcia et al. 1978; Geller et al. 1979b; Goldberg et al. 1964; Haggard et al. 1944), mice (DeCeaurriz et al. 1984; Glowa and Dews 1987; Mashbitz et al. 1936), and baboons (Geller et al. 1979a) exposed by inhalation. Acetone was also neurotoxic in rats after oral exposure, producing prostration (Freeman and Hayes 1985), reduction in nerve conduction velocity (Ladefoged et al. 1989), and excessive salivation (American Biogenics Corp. 1986). Oral exposure to acetone caused prostration in mice (EHRT 1987), weakness, depression, and unconsciousness in rabbits (Walton et al. 1928), and incoordination, staggering, falling, tremors, delirium, prostration, and coma in dogs (Albertoni 1884). No studies were located regarding neurological effects in animals after dermal exposure to acetone. There is no reason to suspect that the effects are route- or species-specific. Acetone is widely distributed throughout the body after absorption, and its presence in the brain of mice after inhalation exposure has been demonstrated (Wigaeus et al. 1982). If enough acetone is absorbed dermally, neurological effects could occur; however, no studies that applied acetone to the skin of animals (Iversen et al. 1981, 1988; Rengstorff et al. 1972, 1976; Ward et al. 1986) described any neurological signs of toxicity.

Epidemiological and Human Dosimetry Studies. A retrospective mortality study of workers exposed to acetone found no significant excess risk of death from any cause (all causes, malignant

neoplasm, circulatory system disease, ischemic heart disease) (Ott et al. 1983a, 1983b). Furthermore, a health evaluation survey of active employees found no evidence of hematological or liver effects (Ott et al. 1983a, 1983c). Other epidemiological studies are limited to studies of pregnancy outcome among female laboratory workers exposed to a variety of solvents, including acetone (Axelsson et al. 1984) and among female factory workers exposed to acetone in Russia (Nizyaeva 1982). No statistically significant increased incidence of miscarriage, perinatal death rate, or malformations of offspring was found in the laboratory workers (Axelsson et al. 1984). Miscarriages and neonates with reduced body weights and length were reported for the factory workers (Nizyaeva 1982), but this study was considered unreliable because of reporting limitations. Acute inhalation studies in volunteers (Matsushita et al. 1969a, 1969b; Nelson,et al. 1943) and on-site medical evaluations of workers on a single workday (Raleigh and McGee et al. 1972; Ross 1973) have found that acetone is irritating to the nose, throat, trachea, lungs, and eyes and may cause unconsciousness if exposure levels are high enough (Ross et al. 1973). Acute experimental studies in humans also found hematological effects, which might also indicate immunological effects (Matsushita et al. 1969a, 1969b) and neurobehavioral effects (Dick et al. 1989; Matsushita et al. 1969a, 1969b). Case reports of patients exposed to acetone vapors by inhalation and/or dermally during and after application of hip casts have found cardiovascular effects (increased pulse rates), gastrointestinal effects (vomiting of blood, hemorrhage), and neurological effects (headache, dizziness, listlessness, mystagmus, difficulty speaking, coma, collapse) (Chatterton and Elliot 1946; Fitzpatrick and Claire 1947; Harris and Jackson 1952; Hift and Pate1 1961; Pomerantz 1950; Renshaw and Mitchell 1956; Strong 1984). No indication that the liver and kidney were target organs, based on clinical chemistry parameters, was found in humans exposed under laboratory conditions (DiVincenzo et al. 1973). Information on effects of acute oral exposure of humans was derived from case reports of accidental or intentional ingestion of acetone and indicate that ingestion causes coma (Gamis and Wasserman 1988; Gitelson et al. 1966; Ramu et al. 1978; Sakata et al. 1989), erosions in the buccal cavity, and possible diabetes-like symptoms (Gitelson et al. 1966). Some of these cases were confounded by coexposure to other narcotic chemicals, such as alcohol and liquid cement. Direct application of acetone to the skin of humans resulted in skin irritation and degenerative changes in the epidermis (Lupulescu et al. 1972, 1973; Lupulescu and Birmingham 1975, 1976), and a case of contact dermatitis was reported (Tosti et al. 1988). Populations likely to be exposed to acetone include workers in industries that process and use acetone, such as, paint, plastic, artificial fibers, and shoe factories. Professional painters and commercial cleaners, laboratory workers, and manicurists, if they still use acetone-containing nail polish remover, can also have greater exposure than the general population. Inhalation and dermal exposure are the

most likely routes of exposure for workers. Cigarette smokers, people living near landfill sites that contain acetone, near highways (automobile exhaust), or incinerators will also have greater exposure primarily by inhalation than the general population. Any epidemiological studies designed for these populations should look for neurological, immunological, hematological, hepatic, renal, reproductive, developmental, and genotoxic effects and cancer. This information would be useful for establishing cause/effect relationships and for future monitoring of individuals living near hazardous waste sites.

Biomarkers of Exposure and Effect

Exposure. Acetone has been identified in breast milk of lactating women, but nursing mothers represent only a fraction of the general population (Pellizzari et al. 1982). Acetone concentrations in expired air, blood, and urine have been monitored in a number of studies of humans exposed to acetone in the workplace (Brugnone et al. 1978, 1980; Fujino et al. 1992; Kawai et al. 1990a, 1992; Pezzagno et al. 1986) as well as by inhalation in controlled laboratory situations (Dick et al. 1989; DiVincenzo et al. 1973; Matsushita et al. 1969a, 1969b; Nomiyama and Nomiyama 1969a, 1969b; Pezzagno et al. 1986; Stewart et al. 1975; Wigaeus et al. 1981), and correlations with exposure levels have been found. Acetone levels in expired air, blood, and urine have also been used to determine whether patients admitted to the hospital in comatose conditions had ingested acetone (Ramu et al. 1978; Sakata et al. 1989). Gastric contents can also be analyzed for determining whether acetone was ingested (Fastlich 1976). In addition, the detection of the odor of acetone in the breath can alert a physician that a patient might have been exposed to acetone (Harris and Jackson 1952; Strong 1944). Carbon dioxide is the main end metabolite of acetone, but has not been used to monitor for exposure, probably because much unchanged acetone is expired and monitoring directly for acetone is more specific. However, acetone is cleared from breath, urine, and blood of humans within l-3 days and shows little tendency to accumulate (Brown et al. 1987; Dick et al. 1989; DiVincenzo et al. 1973; Fukabori et al. 1979; Gamis and Wasserman 1988; Matsushita et al. 1969a, 1969b; Sakata et al. 1989; Wigaeus et al. 1981), so these methods are useful for monitoring only for recent occupational exposure or from accidental or intentional ingestion. Carbon dioxide derived from acetone is also cleared rapidly. This implies that monitoring for more remote exposure is not possible. Other metabolites of acetone enter gluconeogenic pathways, so their origin from exogenous acetone would be masked. Furthermore, because acetone is a metabolite of isopropyl alcohol, it would be difficult to distinguish whether a person was exposed to isopropyl alcohol or acetone. Furthermore, endogenous levels of acetone vary widely due to a variety of factors. As discussed in Section 2.9.3, an abstract indicated

that acetone increased the levels of DNA adducts in rats exposed via drinking water (Cunningham and Gold 1992).

Effect. The consistently observed effect of acetone exposure in animals is the induction of microsomal enzymes, particularly cytochrome P-450IIE1, which can be detected by immunochemical methods using antibodies to it (Johansson et al. 1988). The enzyme induction has been associated with increased liver weights and hepatocellular hypertrophy due to the increased protein content (NTP 1991). Cytochrome P-450IIEl is associated with the metabolism of acetone itself, but acetone is not metabolized to toxic intermediates. However the induction of this enzyme is the mechanism by which acetone potentiates the toxicity of many other chemicals (Brady et al. 1989; Freeman and Hayes 1988; Johansson et al. 1988; Kim et al. 1990; Kobusch et al. 1989; Pankow and Hoffmann 1989; Ronis and Ingelman-Sundberg 1989; Song et al. 1989; Tu et al. 1983; Yoo et al. 1990). Cytochrome P-450IIEl can be induced by a variety of other factors, such as ethanol, fasting, and experimental diabetes (Johansson et al. 1986; Puccini et al. 1990); therefore, the induction is not specific to acetone, and the detection of the enzyme would require liver biopsy and would probably be indicative only of recent exposure. However, an *in vivo* probe to detect elevated levels of cytochrome P-450IIEl in human populations is being developed (see Section 2.9.3). Exposure of rats to acetone has resulted in degeneration of apical microvilli in the kidney (Brown and Hewitt 1984) and nephropathy (NTP 1991), but these effects have not been associated with increased levels of blood urea nitrogen. A strong odor of acetone on the breath and nausea, or high levels of acetone in blood or urine in patients can alert physicians to the possibility of more serious sequelae such as gastrointestinal hemorrhage and narcosis (Harris and Jackson 1952; Strong 1944) or to metabolic acidosis (Gitelson et al. 1966). In rats, acetone exposure resulted in anemia as detected by hematological parameters (American Biogenics Corp. 1986; NTP 1991) and increased testis weight, decreased sperm motility, caudal weight and epididymal weight, and increased incidences of abnormal sperm (NTP 1991). These effects were seen after intermediate-duration exposure. Hematological tests and tests for sperm motility and abnormalities could be used to screen humans for possible hematological effects and effects on fertility. Overt signs of skin irritation could alert physicians to possible degenerative changes, which can be detected by microscopic examination of the epidermis (Lupulescu et al. 1972, 1973). Allergic reactions can be detected by patch testing (Tosti et al. 1988). There does not appear to be a need for additional biomarkers.

Absorption, Distribution, Metabolism, and Excretion. A substantial database exists regarding absorption and excretion of acetone by humans and animals after acute inhalation and oral exposure to acetone (Brown et al. 1987; Charbonneau et al. 1986a, 1986b; Dick et al. 1989; DiVincenzo et al. 1973; Egle 1973; Fukabori et al. 1979; Geller et al. 1979b; Haggard et al. 1944; Hallier et al. 1981; Jakubowski and Wieczorek 1988; Kawai et al. 1992; Landahl and Herrmann 1950; Matsushita et al. 1969a, 1969b; Miller and Yang 1984; Morris 1991; Nomiyama and Nomiyama 1974a; NTP 1988; Pezzagno et al. 1986; Plaa et al. 1982; Price and Rittenberg 1950; Ramu et al. 1978; Sakata et al. 1989; Schrikker et al. 1985, 1989; Skutches et al. 1990; Vangala et al. 1991; Widmark 1919; Wigaeus et al. 1981, 1982) and the information is sufficient to assess relative rates and extent of absorption and excretion. Uptake and excretion are directly proportional to dose and duration of exposure but can be influenced by exercise during exposure and fasting. Acetone does not appear to accumulate to any great extent after repeated exposure, so information on absorption and excretion after intermediate- and chronic-duration exposure does not appear to be necessary. An inhalation study in mice examined the distribution of acetone (Wigaeus et al. 1982), which is widespread regardless of route of uptake. The metabolic pathways of acetone are relatively well understood based on animals studies (Casazza et al. 1984; Hallier et al. 1981; Hetenyi and Ferrarotto 1985; Johansson et al. 1986; Koop and Casazza 1985; Kosugi et al. 1986a, 1986b; Mourkides et al. 1959; Price and Rittenberg 1950; Puccini et al. 1990; Rudney 1954; Sakami and LaFaye 1950, 1951; Skutches et al. 1990). There is some indication that at low doses, the pathways, via methylglyoxal and lactate, predominate, but at higher doses that saturate this pathway, metabolism is shunted to the formate-acetate branch of the propanediol pathway (Kosugi et al. 1986a). Although acetone is absorbed dermally, quantitative data are limited to a study in which an unspecified amount of acetone was applied to the skin of volunteers and levels of acetone were measured in blood, alveolar air, and urine (Fukabori et al. 1979). While the dermal absorption was stated to be fairly rapid, the net amount absorbed and rate of absorption were not determined. Furthermore, inhalation of acetone could not be completely prevented. Additional studies in humans or animals, designed to measure the rate and extent of dermal absorption, would help to fill this gap in understanding the toxicokinetics of acetone. Other than this gap in the database, there appears to be little need for additional information on absorption, distribution, metabolism, and excretion.

Comparative Toxicokinetics. Acetone appears to have similar target organs in animal and humans, such as, the hematological system and the central nervous system. Toxicokinetic studies have been conducted in both humans and animals, especially in humans exposed by inhalation. There appear to be very few differences between animal species, and the dog appears to be a good model for

extrapolating absorption results to humans (DiVincenzo et al. 1973). Metabolic pathways have been elucidated primarily in rats, but mice and rabbits have also been studied. Metabolism involves three different pathways of gluconeogenesis (Casazza et al. 1984; Kosugi et al. 1986a, 1986b). The first step in the metabolism of acetone is dependent on cytochrome P-450IIEl (Casazza et al. 1984), which acetone induces, and this induction has been demonstrated in rats (Johansson et al. 1988), mice (Banhegyi et al. 1988), hamsters (Ueng et a. 1991), and rabbits (Ding and Coon 1990). It appears that the metabolic pathways operate in all species. The distribution of acetone has been studied only in mice exposed by inhalation (Wigaeus et al. 1982). Acetone was widely distributed to organs and tissues throughout the body. This is expected to be true for all species by virtue of its high water solubility, facilitating distribution through the water compartment of the body. There appears to be little need for additional comparative toxicokinetic studies.

Methods for Reducing Toxic Effects. Acetone is readily and passively absorbed from the lungs and gastrointestinal tract into the blood stream (Brown et al. 1987; Charbonneau et al. 1986a; Dick et al. 1989; DiVincenzo et al. 1973; Price and Rittenberg 1959; Sakata et al. 1989; Skutches et al. 1990) and widely to distributed throughout the water compartment (Wigaeus et al. 1982). The only known methods for reducing absorption from the gastrointestinal tract are administration of syrup of ipecac (Stutz and Janucz 1988) and gastric lavage (Sakata et al. 1989). Established methods for reducing body burden are administration of a cathartic, such as magnesium sulfate (Stutz and Janucz 1988) and hemodialysis (Rosansky 1982). The mechanism of the narcotic effects of acetone is not known, but as a solvent, acetone may interfere with the composition of the membranes, altering their permeability to ions (Adams and Bayliss 1968). Developing a method to block this interference might prevent acetone induced coma. Since the metabolites are gluconeogenic precursors, they are probably not toxic. Therefore, acetone itself is probably the toxic agent, and enhancing its metabolism may block its toxic action. However, acetone induces its own metabolism by inducing cytochrome P-450IIEl (Johansson et al. 1986; Puccini et al. 1990). Since ethanol also induces this particular form of P-450IIEl (Johansson et al. 1988; Puccini et al. 1990), the metabolism of acetone might be increased by administering ethanol. Ethanol has been shown to decrease the breath levels of acetone (Jones 1988). However, since acetone potentiates the toxicity of other chemicals by inducing cytochrome P-450IIE1, which enhances the metabolism of the chemicals to reactive intermediates (see Section 2.6), further increasing the cytochrome P-450IIEl levels might be counterproductive in cases of exposure to acetone followed by exposure to the other chemicals. Furthermore, acetone potentiates the central nervous system toxicity of ethanol by inhibiting alcohol dehydrogenase, which is responsible for 90%

of ethanol elimination (Cunningham et al. 1989). Development of other means of enhancing acetone's metabolism might solve this dilemma. The established method for mitigating acute acetone poisoning is the administration of a cathartic, and administration of diazepam to alleviate seizures, or phenobarbital if diazepam is ineffective (Stutz and Janucz 1988). No methods were located to mitigate more subtle effects.

2.9.3 On-going Studies

Dr. C.S. Yang of Rutgers University is conducting on-going studies sponsored by the National Institute of Environmental Health Sciences (NIEHS) on the molecular mechanism of the regulation of cytochrome P-450IIEl (CRISP 1993). The specific aims of the project are to examine the role of acetone, other ketone bodies, and hormones in the induction of this enzyme in rat liver; to characterize the molecular events in the regulation of P-45011El by measuring transcriptional and translational activities and the stabilities of mRNA and proteins; to investigate sex-related differences and hormonal regulation of P-450IIEl in the mouse kidney at the physiological, biochemical, and molecular biological levels; and to understand the active site dynamics and catalytic function of P-450IIEl by examining the structural characteristics of its substrates, by deriving structural information from the amino acid sequence, by modifying specific amino acid residues in the active site with a site-directed mutagenesis approach, and by studying the metabolism and toxicity of environmental chemicals.

Dr. K.E. Thummel of the University of Washington is conducting on-going studies sponsored by the National Institute of General Medical Sciences on whether humans exposed to acetone and other inducers of cytochrome P-450IIE1, or with altered metabolic states, will have elevated levels of this enzyme that can be identified by an *in vivo* probe (CRISP 1993). The utility of the probe candidates will be validated by determining the *in vitro* catalytic specificity of the probe toward human liver P-450IIEl; identifying an *in vivo* metabolic clearance parameter that reflects intrinsic P-450IIEl catalytic activity; and determining whether the *in vivo* clearance parameter predicts direct measurement of hepatic P-450IIEl levels in normal and isoniazid-treated populations.

A number of recent abstracts of studies yet to be published were located covering topics related to toxicokinetics and enzyme induction, These studies are described below.

In rats treated intraperitoneally with acetone, the concentration of acetone in expired air was 40% and the half-life was 4 hours (Teramoto et al. 1989).

Fed pregnant rats on gestational day 20 had slightly higher endogenous serum acetone levels compared with nonpregnant controls, and acetone monooxygenase activity was significantly lower in the pregnant rats than in the nonpregnant rats (Casazza et al. 1990). Administration of acetone in the drinking water for 5 days increased the acetone monooxygenase activity, but the activity was lower than in acetone treated nonpregnant rats. Immunoblot analysis showed that cytochrome P-45OIIEl decreased progressively during pregnancy and rapidly returned to normal after parturition. The decrease in P-45011El was accompanied by a gradual decrease in P-450IIEl mRNA. The results indicated that liver P-45011El is suppressed in the pregnant rat, while the levels still respond to acetone induction. A pretranslational mechanism was suggested for P-450IIEl suppression.

Serum levels of endogenous acetone were 26 μmol/mL in 20-day pregnant rats and 14 μmol/mL in the pooled blood from litters of fetal siblings (Casazza et al. 1988). No increase in maternal blood acetone was seen on day postpartum day 1, but the levels in pups were markedly increased. Measurable levels of serum acetol and 1,2-propanediol were also found in the 2- to 3-day-old pups. Injection of acetol into the pups resulted in a nine-fold increase in 1,2-propanediol levels. The results demonstrate the acetone is normally present in newborn rats, and that the pups are able to metabolize acetone to 1,Zpropanediol soon after birth.

Acetone added to the culture system increased the incorporation of methionine into cytochrome P-450IIEl in rabbit hepatocytes, indicating that the acute phase of acetone induction of this enzyme partly involves an increased rate of P-450IIEl protein synthesis (Kraner et al. 1991).

Xenobiotic metabolizing activities associated with cytochrome P-45011El were elevated in liver microsomes from acetone treated rats (Menez et al. 1990). Coadministration of phorbol myristate acetate, an activator of protein kinase c, with acetone suppressed P-450IIEl content and the activities of the associated oxidases, indicating that the induction of P-450IIEl by acetone involves inhibition of protein kinase c.

Acetone inhibited the tumor promoting activity of phorbol myristate acetate following initiation with dimethylbenz[a]anthracene on mouse skin (Weiss et al. 1988).

N-nitrosodimethylamine demethylase activity in rat hepatoma was increased by treating the rats with acetone (Hu et al. 1990).

The levels of hepatic N7-methylguanine and O6-methylguanine DNA adducts were significantly increased by treatment of rats with 10% acetone in drinking water for 10 days (Cunningham and Gold 1992).

3. CHEMICAL AND PHYSICAL INFORMATION

3.1 CHEMICAL IDENTITY

Data pertaining to the chemical identities of acetone, the simplest aliphatic ketone, are listed in Table 3-l.

3.2 PHYSICAL AND CHEMICAL PROPERTIES

The physical and chemical properties of acetone are given in Table 3-2. The physical properties of acetone, such as high evaporation rate, low viscosity, and miscibility with water and several organic solvents make it suitable for use as a solvent (Krasavage et al. 1982). Because of its ability to undergo addition, oxidation/reduction, and condensation reactions, acetone is used as a raw material in the chemical synthesis of many commercial products (Nelson and Webb 1978).

TABLE 3-1. Chemical Identity of Acetone

Characteristic	Information	Reference
Chemical name	Acetone	HSDB 1992
Synonym(s)	Dimethyl ketone; 2-propanone; beta-ketopropane	HSDB 1992
Registered trade name(s)	No data	
Chemical formula	C_3H_6O	
Chemical structure	$CH_3\text{-}\overset{\overset{\displaystyle O}{\|}}{C}\text{-}CH_3$	
Identification numbers:		
CAS registry	67-64-1	HSDB 1992
NIOSH RTECS	AL3150000	HSDB 1992
EPA hazardous waste	U002; F003	HSDB 1992
OHM/TADS	7216568	HSDB 1992
DOT/UN/NA/IMCO shipping	UN1090	HSDB 1992
HSDB	41	HSDB 1992
NCI	ND	HSDB 1992

CAS = Chemical Abstracts Services; DOT/UN/NA/IMCO = Department of Transportation/United Nations/North America/International Maritime Dangerous Goods Code; EPA = Environmental Protection Agency; HSDB = Hazardous Substances Data Bank; NCI = National Cancer Institute; NIOSH = National Institute for Occupational Safety and Health; OHM/TADS = Oil and Hazardous Materials/Technical Assistance Data System; RTECS = Registry of Toxic Effects of Chemical Substances

TABLE 3-2. Physical and Chemical Properties of Acetone

Property	Information	Reference
Molecular weight	58.08	Riddick et al. 1986
Color	Colorless	HSDB 1992
Physical state	Liquid	HSDB 1992
Melting point	-95.35°C	HSDB 1992
Boiling point	56.2°C at 1 atm	HSDB 1992
Density:		
at 20°C	0.78998 g/mL	Riddick et al. 1986
at 25°C	0.78440 g/mL	Riddick et al. 1986
at 30°C	0.78033 g/mL	Riddick et al. 1986
Odor	Mildly pungent and aromatic	Nelson and Webb 1978
Odor threshold:		
Water	20 ppm (w/v)	Amoore and Hautala 1983
Air (absolute)	13–20 ppm (v/v)	Amoore and Hautala 1983; Hellman and Small 1974
100% odor recognition	100–140 ppm	Hellman and Small 1974; Leonardos et al. 1969
Solubility:		
Water at 20°C	Completely miscible	HSDB 1992
Organic solvent(s)	Soluble in benzene and ethanol	HSDB 1992
Partition coefficients:		
Log K_{ow}	-0.24 (recommended value)	Collander 1951; Sangster 1989
Log K_{oc}	0.73 (estimated)[a]	Lyman 1982
Vapor pressure at 20°C	181.72 mmHg	Riddick et al. 1986
Henry's law constant:		
at 25°C	4.26×10^{-5} atm·m^3/mol	Rathbun and Tai 1987
Autoignition temperature	465°C	HSDB 1992
Flashpoint (closed cup)	-20°C	HSDB 1992
Flammability limits in air at 25°C	Lower, 2.15%; upper, 13.0%	HSDB 1992
Conversion factors:		
in air at 25°C	1 ppm = 2.374 mg/m^3	HSDB 1992
Explosive limits	Lower, 2.6% in air (v/v); upper, 12.8% in air (v/v)	HSDB 1992 HSDB 1992

[a]Estimated by regression equation 4-13 in Lyman (1982)

w/v = weight per volume; v/v = volume per volume

4. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

4.1 PRODUCTION

In 1991, 12 companies at 14 locations produced acetone in the United States. The U.S. manufacturers of acetone in 1991, their respective manufacturing sites, and production capacities are shown in Table 4-l (SRI 1992).

Exxon Chemical Americas also produced acetone in the United States in 1990 (USITC 1991).
Table 4-2 lists the companies that process acetone and the intended use and maximum amounts of the chemical stored on site.

The reported total production volume of acetone in the United States was 2,330 million pounds in 1990 (USITC 1991). Historically, the reported growth of acetone demand was 2.1% per year during the 1980's and is expected to grow at a rate of 2.5% per year through 1994 (CMR 1990).

Most acetone is manufactured by one of two processes, cumene peroxidation or isopropanol dehydrogenation (SRI 1991). In the peroxidation process, cumene is oxidized to hydroperoxide, which is cleaved to yield acetone and phenol. In the dehydrogenation process, isopropanol is catalytically dehydrogenated to yield acetone and hydrogen (Nelson and Webb 1978). Relatively small quantities of acetone are recovered as a byproduct from the production of hydroquinone and oxidation of propylene oxide (SRI 1991).

4.2 IMPORT/EXPORT

The amount of acetone exported by the United States has increased in recent years. Export volume in 1987 was 5% of total production volume (CMR 1987). By 1990, export volume had increased to an estimated 138 million pounds, or 6% of the 2,330 million pounds produced (CMR 1990). The United States imported 110 million pounds of acetone in 1989, and import volume is expected to increase through 1994, due to a projected increase in domestic demand.

TABLE 4-1. U.S. Manufacturers of Acetone in 1991[a]

Manufacturer	Location	Annual capacity, (million of pounds)
Allied Signal, Inc.	Philadelphia, PA	490
Aristech Chemical Corp.	Haverhill, OH	392
ARCO Chemical Co.	Bayport, TX	62
BTL Speciality Resins Corp.	Blue Island, IL	53
Dow Chemical U.S.A.	Oyster Creek, TX	335
Eastman Chemical Co.	Kingsport, TN	25
General Electric Co.	Mount Vernon, IN	380
Georgia Gulf Corp.	Pasadena, TX	100
	Plaquemine, LA	2,656
The Goodyear Tire & Rubber Co.	Bayport, TX	12
Shell Oil Co.	Deer Park, TX	459
	Wood River, IL	350
Texaco, Inc.	El Dorado, KS	55
Union Carbide Corp.	Institute, WV	170
Total		3,148

TABLE 4-2. Facilities That Manufacture or Process Acetone[a]

State[b]	Number of facilities	Range of maximum amounts on site in thousands of pounds[c]	Activities and uses[d]
AK	1	0.1-0.9	1, 5
AL	46 (1)[e]	0-9,999	1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13
AR	50 (4)[e]	0-999	1, 2, 3, 5, 6, 8, 9, 11, 12, 13
AZ	24 (2)[e]	0-999	1, 3, 5, 8, 11, 12, 13
CA	220 (13)[e]	0-499,999	1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13
CO	17	0-999	8, 11, 12, 13
CT	27 (5)[e]	0.1-9,999	1, 2, 3, 5, 7, 8, 9, 11, 12, 13
DE	12 (1)[e]	0.1-99	2, 3, 7, 9, 10, 11, 12, 13
FL	107 (3)[e]	0-999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
GA	80 (10)[e]	0-999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
HI	1	1-9	13
IA	36 (2)[e]	0.1-999	1, 2, 3, 7, 8, 9, 10, 11, 12, 13
ID	9	1-99	1, 2, 3, 6, 12, 13
IL	110 (7)[e]	0.1-99,999	1, 3, 4, 7, 8, 9, 10, 11, 12, 13
IN	132 (2)[e]	0-49,999	1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13
KS	34 (2)[e]	0.1-9,999	1, 3, 4, 8, 10, 11, 12, 13
KY	41 (3)[e]	0.1-9,999	1, 5, 7, 8, 10, 11, 12, 13
LA	37 (1)[e]	0-499,999	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13
MA	66 (5)[e]	0-999	2, 3, 8, 9, 10, 11, 12, 13
MD	17 (1)[e]	0.1-99	1, 2, 3, 5, 8, 9, 11, 13
ME	23 (2)[e]	0-999	1, 2, 3, 5, 6, 7, 8, 9, 11, 12, 13
MI	140 (5)[e]	0-49,999	1, 2, 3, 5, 7, 8, 9, 10, 11, 12, 13
MN	63 (2)[e]	0.1-999	1, 2, 3, 5, 7, 8, 9, 11, 12, 13
MO	71	0.1-99,999	1, 2, 3, 5, 7, 8, 9, 10, 11, 12, 13
MS	31 (3)[e]	0-999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13
MT	1	1-9	1, 5
NC	154 (5)[e]	0-9,999	1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13
ND	2	0.1-99	12, 13
NE	17	0-99	8, 11, 12, 13
NH	16 (1)[e]	0.1-99	1, 2, 3, 6, 8, 9, 11, 12, 13
NJ	98 (4)[e]	0-9,999	1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 13
NM	8	0.1-99	12, 13
NV	3 (1)[e]	1-99	8, 11, 13
NY	89 (6)[e]	0.1-999	1, 2, 3, 5, 7, 8, 9, 10, 11, 12, 13
OH	157 (5)[e]	0-99,999	1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 13
OK	13	0.1-99	2, 3, 8, 10, 11, 12, 13
OR	46 (2)[e]	0-999	1, 5, 6, 7, 8, 9, 10, 11, 12, 13
PA	130 (9)[e]	0-9,999	1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 13
PR	25	1-9,999	2, 3, 4, 7, 8, 10, 11, 12, 13
RI	18 (1)[e]	1-99	1, 5, 9, 10, 11, 12, 13
SC	55 (1)[e]	0-9,999	1, 2, 3, 4, 5, 8, 9, 11, 12, 13
SD	3	1-9	13

TABLE 4-2 Facilities That Manufacture or Process Acetone[a] *(Continued)*

State[b]	Number of facilities	Range of maximum amounts on site in thousands of pounds[c]	Activities and uses[d]
TN	83 (2)[e]	0-49,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
TX	166 (4)[e]	0-99,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
UT	16	0-99	3, 8, 10, 11, 12, 13
VA	81 (2)[e]	0-9,999	1, 2, 3, 5, 8, 9, 10, 11, 12, 13
VT	4 (1)[e]	1-99	8, 12, 13
WA	65 (5)[e]	0-999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
WI	75 (5)[e]	0-99,999	1, 2, 3, 5, 7, 8, 9, 10, 11, 12, 13
WV	16	0-49,999	1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13

[a]Derived from TRI90 (1992)
[b]Post office state abbreviations
[c]Data in TRI are maximum amounts on site at each facility.
[d]Activities/Uses:

1. produce
2. import
3. for on-site use/processing
4. for sale/distribution
5. as a byproduct
6. as an impurity
7. as a reactant
8. as a formulation component
9. as an article component
10. for repackaging only
11. as a chemical processing aid
12. as a manufacturing aid
13. ancillary or other use

[e]Number of facilities reporting "no data" regarding maximum amount of the substance on site

4.3 USE

Acetone is used primarily as an intermediate in chemical production and as a solvent (Nelson and Webb 1978). In 1989, 40% of the available acetone was used in the production of methyl methacrylate, methacrylic acid, and higher methacrylates; 20% was used as a solvent; 13% was used in the production of bisphenol A; 10% was used in the production of methyl isobutyl ketone and methyl isobutyl carbinol; 6% was used in drug and pharmaceutical applications; 5% accounted for miscellaneous uses; and 6% was exported (CMR 1990).

4.4 DISPOSAL

A small amount of acetone is regenerated from solvent wastes produced during its use by reclaiming processes (Kupferschmid and Perkins 1986). Acetone can be removed from waste water by air stripping (HSDB 1992), but the vapor phase acetone generated during stripping requires a suitable disposal method. The three methods commonly used for the disposal of waste containing acetone are underground injection, burial in sanitary landfill, and incineration. The underground injection of acetone-containing waste is allowed under the amended Section 148.10 of Code of Federal Regulations (EPA 1991b). The land disposal of waste waters containing spent acetone is allowed under Section 268.41 of the Code of Federal Register as long as the concentrations of acetone and other permissible spent cosolvents in the waste do not exceed 0.05 and 0.59 mg/L, respectively (EPA 1988a). Incineration under controlled conditions (to attain complete combustion) is one of the better methods of disposal for acetone, and incineration is easier when acetone is mixed with a more flammable solvent. The suitable methods for the destruction of acetone are fluidized bed incineration at a temperature of 450-980°C and residence times of seconds or rotary kiln incineration at 820-1,600ºC and residence times of seconds (HSDB 1992).

5. POTENTIAL FOR HUMAN EXPOSURE

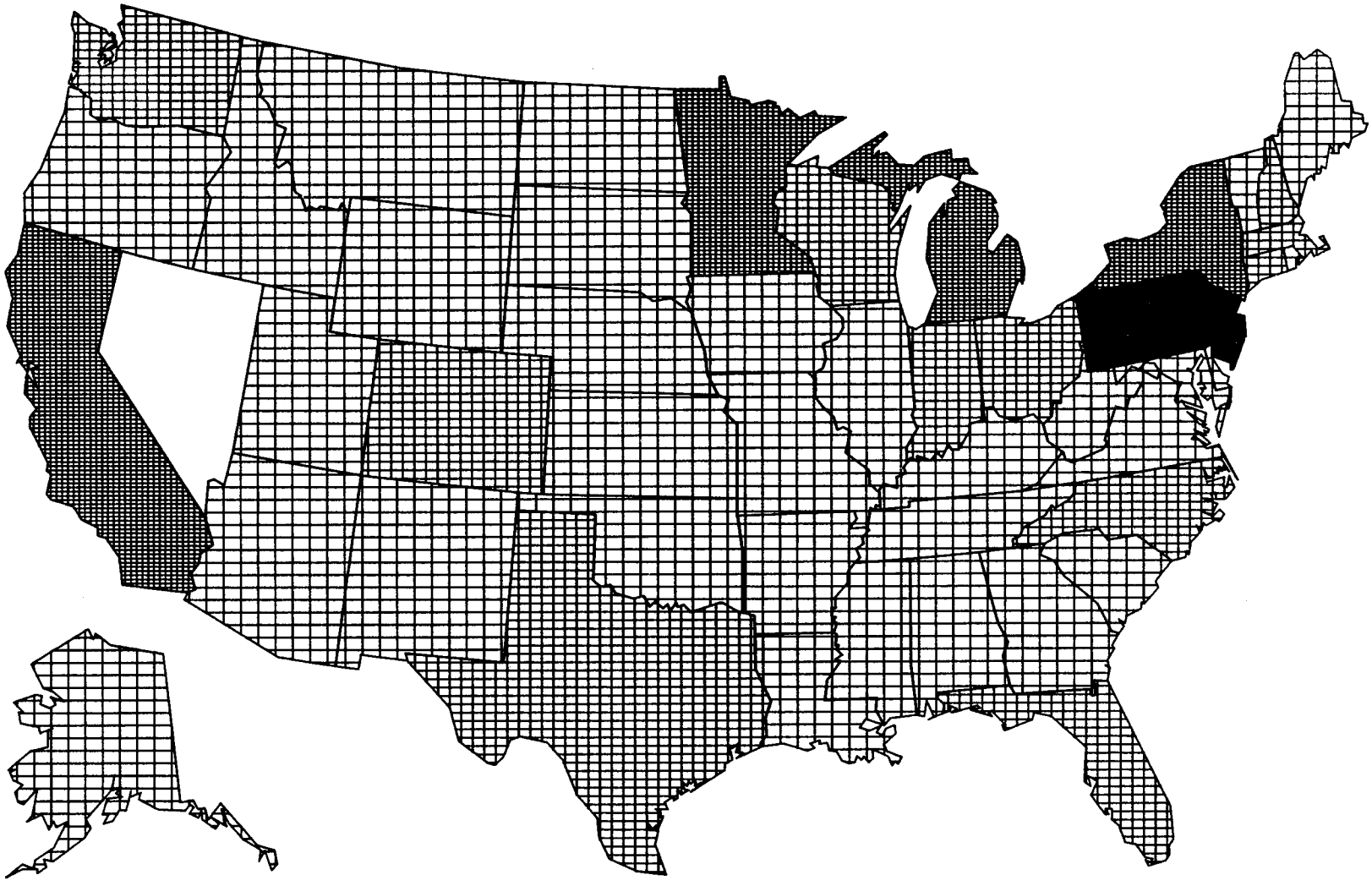
5.1 OVERVIEW

Acetone is emitted into the atmosphere both from natural and anthropogenic (man-made) sources. Natural sources of emission include plants and trees (Graedel et al. 1986; Isidorov et al. 1985; Khalil and Rasmussen 1992), volcanic eruptions (Isidorov et al. 1990), forest fires (Graedel et al. 1986), and insects and microbes (Graedel et al. 1986). Acetone is also produced endogenously and expired in human breath (Conkle et al. 1975). Some important anthropogenic sources of acetone in the air include vehicular exhaust (Graedel et al. 1986), chemical manufacturing (Graedel et al. 1986), tobacco smoke (Manning et al. 1983), wood burning and pulping (Graedel et al. 1986), refuse and polyethylene combustion (Graedel et al. 1986; Hodgkin et al. 1982; NAS 1976), petroleum production (Graedel et al. 1986), certain landfill sites (Hodgson et al. 1992; LaRegina et al. 1986), and solvent use (Graedel et al. 1986). The sensitized photoreaction of dissolved organic matters naturally produces acetone in seawater (Mopper and Stahovec 1986). Chemical manufacturing industries (Abrams et al. 1975), energy-related industries (Mohr and King 1985), and user industries (Abrams et al. 1975) release acetone to surface waters. Acetone is released into groundwater mainly as a result of leaching from municipal and industrial landfills (Brown and Donnelly 1988). The principal sources of acetone in soil are municipal and industrial discharge in landfills (EPA 1988b). Another source is atmospheric deposition (Grosjean and Wright 1983). Acetone is released in soil from natural sources, such as disposed agricultural and food wastes and animal wastes (Graedel et al. 1986).

Acetone has been identified in at least 560 of the 1,350 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (HAZDAT 1991). However, the number of sites evaluated for acetone is not known. The frequency of these sites within the United States can be seen in Figure 5-l. Of these sites, 559 are located in the United States and 1 is located in the Commonwealth of Puerto Rico (not shown).

The two processes that are important in determining the fate of acetone in the atmosphere are reaction with hydroxyl radicals and photolysis. The estimated half-life of acetone in the air due to combinations of these two reactions is 22 days (Meyrahn et al. 1986). Because of this reasonably long half-life, acetone is transported long distances from its source of emission. Wet deposition transports

FIGURE 5-1. FREQUENCY OF NPL SITES WITH ACETONE CONTAMINATION *



FREQUENCY
1 TO 8 SITES
9 TO 24 SITES
25 TO 39 SITES
46 TO 51 SITES

*Derived from HAZDAT 1991

atmospheric acetone to surface water and the terrestrial surface (Grosjean and Wright 1983). The most important fate determining process for acetone in water is biodegradation (Rathbun et al. 1982). Because of its high water solubility, acetone does not adsorb significantly to sediment and suspended solids in water. Acetone does not bioconcentrate in aquatic organisms (Rustung et al. 1931), and there is no data on acetone biomagnification in aquatic and terrestrial food chains. Biodegradation is the most important degradative process for acetone in sediment and soil (Rathbun et al. 1982). The important transport processes of acetone in soil are volatilization to the atmosphere and leaching into groundwater.

The levels of acetone in ambient air and water are generally low. The concentration of acetone in the atmosphere in remote areas is ≤1 ppb (volume per volume [v/v]) (1 ppb=0.00l ppm) (Cavanagh et al. 1969; Arnold et al. 1986). Its mean concentration in the atmosphere of rural areas is <3 ppb (Shepson et al. 1991; Snider and Dawson 1985). The mean concentration of acetone in urban air in the United States is 6.9 ppb (Shah and Singh 1988). Acetone concentration in indoor air in the United States is generally slightly higher than outdoor air (8.0 ppb versus 6.9) (Shah and Singh 1988), due to the use of household consumer products containing acetone. The concentration of acetone in open seawater near the Bahamas was 0.35 ppb (Kieber and Mopper 1990). The concentration of acetone in the Potomac River in Virginia was below the detection limit of 40 ppb (Hall et al. 1987), and the level will be higher in water receiving industrial and municipal discharge containing acetone. An industrial landfill leachate in Michigan contained 62 ppm acetone (Brown and Donnelly 1988). An acetone concentration ≤3,000 ppb was detected in a drinking water well in New Jersey (Burmaster 1982). The level of acetone in finished drinking water is generally low (Coleman et al. 1976; Keith et al. 1976), and the reported concentration in drinking water from Seattle, Washington, was 1 ppb (Keith et al. 1976). A concentration of 6 ppb acetone was detected in the sediment of a creek adjacent to a landfill in Louisville, Kentucky (Stonebraker and Smith 1980). Acetone has been detected in the volatile components of several fruits and vegetables (Bartley and Schwade 1989; Lovegren et al. 1979).

The general population is exposed to acetone by inhaling ambient air and by ingesting drinking water and food containing acetone. No data for the total daily intake of acetone for the general population were located. There is a great deal of evidence that workers in certain industries, such as certain paint, plastic, artificial fiber, and shoe factories are exposed to much higher levels of acetone than the general population (Kawai et al. 1990a; Pezzagno et al. 1986). Professional painters, and commercial and household cleaners are also likely to be exposed to higher acetone concentrations than the general

population. Among the general population, smokers, frequent users of nail polish removers and people who live near certain landfill sites (emitting higher than ambient levels of acetone) or other industrial sources of emission are susceptible to higher exposure concentrations of acetone.

5.2 RELEASES TO THE ENVIRONMENT

5.2.1 Air

Acetone is emitted into the atmosphere both from natural and anthropogenic (man-made) sources. Natural sources of emission include plants and trees. Acetone has been detected in a number of plant volatiles including onions, grapes, cauliflower, tomatoes, wild mustard, beans, and peas (HSDB 1992), and emissions have been detected from a variety of trees including willow, aspen, birch, balsam poplar, oak, fir, pine, juniper, cedar, and cypress (Isidorov et al. 1985; Khalil and Rasmussen 1992). Acetone is produced endogenously and released as a component of human breath (Conkle et al. 1975; Krotoszynski 1977; Trotter et al. 1971). Volatiles from animal wastes, microbes, and insects are also examples of natural sources of acetone in the air (Graedel et al. 1986). In addition, forest fires and volcanic eruptions emit acetone into the atmosphere (Graedel et al. 1986; Isidorov et al. 1990).

Some important anthropogenic sources of acetone in the air are automobile and diesel exhaust (Barber and Lodge 1963; Jonsson et al. 1985; Lloyd 1978), chemical manufacture (Graedel et al. 1986), tobacco smoke (Manning et al. 1983), wood burning and pulping (Graedel et al. 1986; Kleinienst et al, 1986; Lipari et al. 1984), polyethylene burning (Hodgkin et al. 1982), refuse combustion (NAS 1976), petroleum production (Graedel 1978), certain landfill sites (Hodgson et al. 1992; LaRegina et al. 1986; Militana and Mauch 1989), and solvent uses (Medinilla and Espigares 1988). Acetone is also formed in the atmosphere from the photochemical oxidation of propane (Arnold and Ziereis 1986; Singh and Hanst 1981) and possibly from propylene oxide and epichlorohydrin (Spicer et al. 1985). Atmospheric emissions are also likely from several consumer products including nail polish removers, particle board (Tichenor and Mason 1988), carpet backing (Hodgson et al. 1993), some paint removers (Hahn and Werschulz 1986), and a number of liquid/paste waxes or polishes (Knoeppel and Schauenburg 1989; Sack et al. 1992). Certain detergents/cleansers (Knoeppel and Schauenburg 1989; Sack et al. 1992), adhesives, and carburetor and choke cleaners (EPA 1989) are also known to contain acetone.

Acetone released into air from facilities in each state in the United States that manufactured or processed acetone during 1990 are listed in Table 5-l (TR190 1992). According to TR190 (1992), an estimated total of 180 million pounds of acetone, amounting to 96.7% of the total environmental release, was discharged to the air from manufacturing and processing facilities in the United States in 1990. The TRI data should be used with caution since only certain types of facilities were required to report. This is not an exhaustive list. From the monitoring data on levels of acetone in pristine areas and urban/suburban areas (see Section 5.4.1), it seems likely that the total emission of acetone in the atmosphere from anthropogenic sources exceeds the total emission from natural sources.

5.2.2 Water

Acetone is released into surface water as waste water from certain chemical manufacturing industries (Gordon and Gordon 1981; Hites and Lopez-Avila 1980; Jungclaus et al. 1978). It is also released in water from energy-related industries, such as coal-gasification (Mohr and King 1985; Pellizzari et al. 1979) and oil shale processing (Hawthorne and Sievers 1984; Pellizzari et al. 1979). Acetone was found in 27 of 63 effluent waters from a wide range of chemical industries in the United States (Perry et al, 1979). A survey of industrial effluents indicates that acetone was detected in effluents from various industrial products such as, paper, plastic, pharmaceutical, specialty cleaning and polishing products, paint and allied products, gum and wood chemicals, cyclic intermediates, industrial organic chemicals, gypsum products, and paper board products (Abrams et al. 1975).

Acetone is released to groundwater as a result of leaching from municipal and industrial landfills (Brown and Donnelly 1988; Gould et al. 1983; Sawhney and Raabe 1986; Steelman and Ecker 1984; Stonebraker and Smith 1980). Leaching from polyethylene distribution pipes may be a source of acetone in drinking water (Anselme et al. 1985). One of the sources of acetone in seawater is the sensitized photoreaction of dissolved organic matters (Mopper and Stahovec 1986). The releases of acetone to water from facilities that manufactured or processed acetone in states within the United States during 1990 are reported in Table 5-l (TR190 1992). According to TR190 (1992), an estimated total of 1.28 million pounds of acetone, amounting to 0.7% of the total environmental release, was discharged to the water from manufacturing and processing facilities in the United States in 1990. In addition, these facilities discharged an estimated 12.2 million pounds of acetone, amqunting to 6.6% of the total environmental release to municipal waste water of publicly owned treatment works (POTW) in 1990 (TR190 1992).

TABLE 5-1. Releases to the Environment from Facilities That Manufacture or Process Acetone[a]

		Range of reported amounts released in thousands of pounds[b]						
State[c]	Number of facilities	Air	Underground injection	Water	Land	Total Environment[d]	POTW[e] transfer	Off-site waste transfer
AK	1	22.5-22.5	0.0-0.0	2.5-2.5	0.0-0.0	25.0-25.0	0.0-0.0	0.0-0.0
AL	46	0.0-240.6	0.0-0.0	0.0-7.8	0.0-16.0	0.0-240.9	0.0-0.3	0.0-30.7
AR	50	0.3-540.0	0.0-0.0	0.0-14.9	0.0-0.3	0.3-540.0	0.0-0.0	0.0-41.3
AZ	24	0.0-1835.1	0.0-0.0	0.0-0.0	0.0-49.6	0.0-1835.1	0.0-43.0	0.0-68.5
CA	220	0.0-160.3	0.0-0.0	0.0-63.0	0.0-0.0	0.0-160.3	0.0-56.5	0.0-132.0
CO	17	0.0-228.1	0.0-0.0	0.0-0.0	0.0-0.0	0.0-228.1	0.0-12.9	0.0-38.2
CT	27	0.0-81.2	0.0-0.0	0.0-400.0	0.0-0.0	0.0-414.6	0.0-378.9	0.0-178.0
DE	12	0.1-71.7	0.0-0.0	0.0-0.0	0.0-0.0	0.1-71.7	0.0-40.5	0.0-41.1
FL	107	0.0-1114.6	0.0-64.0	0.0-6.3	0.0-0.3	0.1-1114.6	0.0-397.3	0.0-198.3
GA	80	0.0-950.0	0.0-0.0	0.0-17.0	0.0-0.8	0.0-952.2	0.0-290.0	0.0-201.5
HI	1	9.7-9.7	0.0-0.0	0.0-0.0	0.0-0.0	9.7-9.7	0.0-0.0	0.0-0.0
IA	36	0.0-490.0	0.0-0.0	0.0-0.3	0.0-0.2	0.0-490.0	0.0-0.8	0.0-14.8
ID	9	1.0-57.0	0.0-0.0	0.0-7.1	0.0-5.7	1.0-57.9	0.0-0.3	0.0-37.2
IL	110	0.0-862.0	0.0-0.0	0.0-6.0	0.0-0.3	0.0-862.0	0.0-302.6	0.0-259.5
IN	132	0.0-2480.0	0.0-0.0	0.0-0.9	0.0-0.3	0.0-2,480.0	0.0-190.0	0.0-2600.0
KS	34	0.0-330.0	0.0-0.0	0.0-7.0	0.0-0.0	0.0-337.0	0.0-92.8	0.0-39.6
KY	41	0.1-760.0	0.0-0.0	0.0-4.7	0.0-0.3	0.1-760.0	0.0-0.3	0.0-191.4
LA	37	0.0-128.0	0.0-1500.0	0.0-17.1	0.0-0.5	0.0-1510.9	0.0-0.0	0.0-70.5
MA	66	0.0-430.6	0.0-0.0	0.0-21.7	0.0-4.3	0.0-430.6	0.0-6.5	0.0-233.7
MD	17	0.0-57.0	0.0-0.0	0.0-0.0	0.0-0.0	0.0-57.0	0.0-30.0	0.0-54.1
ME	23	1.1-165.4	0.0-0.0	0.0-11.0	0.0-4.6	1.3-165.4	0.0-1.3	0.0-20.8
MI	140	0.0-824.0	0.0-775.9	0.0-7.5	0.0-1.5	0.0-1553.3	0.0-748.0	0.0-104.2
MN	63	0.3-175.6	0.0-0.0	0.0-19.0	0.0-0.1	0.3-175.6	0.0-25.4	0.0-62.1
MO	71	0.1-402.2	0.0-0.0	0.0-0.0	0.0-0.3	0.1-402.2	0.0-53.0	0.0-83.0
MS	31	0.0-435.2	0.0-0.0	0.0-13.0	0.0-0.1	0.0-435.2	0.0-0.0	0.0-7.3
MT	1	5.9-5.9	0.0-0.0	6.8-6.8	0.0-0.0	12.7-12.7	0.0-0.0	0.0-0.0
NC	154	0.0-503.4	0.0-0.0	0.0-9.3	0.0-0.3	0.0-503.4	0.0-141.0	0.0-60.0
ND	2	10.1-16.5	0.0-0.0	0.0-0.0	0.0-0.0	10.1-16.5	0.0-0.0	0.0-0.0
NE	17	0.2-68.2	0.0-0.0	0.0-0.0	0.0-0.0	0.2-68.2	0.0-0.0	0.0-15.9
NH	16	0.0-59.2	0.0-0.0	0.0-4.2	0.0-8.5	0.5-59.2	0.0-0.0	0.0-16.2
NJ	98	0.0-931.5	0.0-0.0	0.0-1.2	0.0-48.3	0.0-931.5	0.0-318.4	0.0-570.2
NM	8	1.0-53.0	0.0-0.0	0.0-0.0	0.0-0.3	1.0-53.0	0.0-0.0	0.0-2.4
NV	3	1.3-81.9	0.0-0.0	0.0-0.0	0.0-0.0	1.3-81.9	0.0-0.0	0.0-1.5
NY	89	0.0-2730.0	0.0-0.0	0.0-21.0	0.0-0.8	0.0-2730.3	0.0-340.0	0.0-456.7
OH	157	0.0-1369.0	0.0-630.0	0.0-4.3	0.0-3.7	0.0-1955.0	0.0-75.0	0.0-632.4
OK	13	0.5-165.2	0.0-0.0	0.0-0.0	0.0-0.0	0.5-165.2	0.0-1.7	0.0-46.4
OR	46	0.3-1305.8	0.0-0.0	0.0-7.5	0.0-0.3	0.3-1305.8	0.0-93.7	0.0-115.5
PA	130	0.0-335.1	0.0-0.0	0.0-2.5	0.0-2.5	0.0-335.1	0.0-1894.0	0.0-190.3
PR	25	0.0-1342.4	0.0-0.0	0.0-0.0	0.0-0.1	0.0-1342.4	0.0-201.6	0.0-153.2

TABLE 5-1. Releases to the Environment from Facilities That Manufacture or Process Acetone[a] *(Continued)*

State[c]	Number of facilities	Range of reported amounts released in thousands of pounds[b]						
		Air	Underground injection	Water	Land	Total Environment[d]	POTW[e] transfer	Off-site waste transfer
RI	18	0.0-233.7	0.0-0.0	0.0-6.1	0.0-0.0	0.0-233.7	0.0-0.0	0.0-302.9
SC	55	0.0-2921.8	0.0-0.0	0.0-12.0	0.0-1.6	0.0-2923.8	0.0-1.7	0.0-301.8
SD	3	1.4-32.0	0.0-0.0	0.0-0.0	0.0-0.0	1.4-32.0	0.0-0.0	1.6-6.3
TN	83	0.3-31100.0	0.0-0.0	0.0-42.8	0.0-8.9	0.3-31123.0	0.0-47.2	0.0-85.0
TX	166	0.0-5223.8	0.0-326.0	0.0-9.0	0.0-5.9	0.0-5223.8	0.0-380.0	0.0-462.8
UT	16	0.0-98.2	0.0-0.0	0.0-0.0	0.0-0.0	0.0-98.2	0.0-7.2	0.0-6.6
VA	81	0.0-10530.0	0.0-0.0	0.0-3.8	0.0-5.4	0.0-10535.7	0.0-1889.7	0.0-60.6
VT	4	0.0-41.9	0.0-0.0	0.0-0.0	0.0-0.0	0.0-41.9	0.0-0.0	0.0-10.4
WA	65	0.0-404.2	0.0-0.0	0.0-12.0	0.0-2.7	0.0-404.2	0.0-1.5	0.0-93.1
WI	75	0.0-222.2	0.0-0.0	0.0-2.2	0.0-0.0	0.0-222.2	0.0-42.4	0.0-268.0
WV	16	0.1-3218.1	0.0-0.0	0.0-10.0	0.0-18.7	0.1-3222.4	0.0-483.0	0.0-116.9

[a]Derived form TRI90 (1992)
[b]Data in TRI are maximum amounts released by each facility. Quantities reported here have been rounded to the nearest hundred pounds, except those quantities >1 million pounds, which have been rounded to the nearest thousand pounds.
[c]Post office state abbreviation
[d]The sum of all releases of the chemical to air, land, water, and underground injection wells by a given facility
[e]POTW = publicly owned treatment works

5.2.3 Soil

Acetone leaches readily in soil (see Section 5.4.3). Therefore, the detection of acetone in leachate and groundwater from municipal and industrial landfills indicates the source of acetone in landfill soils is municipal and industrial discharge. Information regarding the release of acetone from facilities that manufactured or processed the compound in 1990 is reported in Table 5-l (TR190 1992). Table 5-l shows that the amount of acetone released into soil from these facilities accounts for 0.1% of the total environmental release of acetone. Other sources of acetone released into soil include disposal of agricultural and food waste, animal wastes (see Section 5.2.1), and atmospheric wet deposition. Household septic tank effluents are another source of acetone in soil because these effluents containing acetone are discharged into the soil (DeWalle et al. 1985). According to the HAZDAT of ATSDR, acetone was detected in 43% of the soil from Superfund sites tested for acetone (HAZDAT 1991). The information used from the HAZDAT includes data from NPL sites only.

5.3 ENVIRONMENTAL FATE

5.3.1 Transport and Partitioning

Organic compounds with ambient vapor pressure $>10^{-4}$ mmHg should exist almost entirely in the vapor phase (Eisenreich et al. 1981). Since the vapor pressure of acetone is 181.72 mmHg at 20°C (see Table 3-2), acetone should exist exclusively in the vapor phase in the atmosphere. Furthermore, the collection methods used for the quantification of acetone in the atmosphere (Jarke et al. 1981; Juttner 1986; LaRegina et al. 1986) indicate that atmospheric acetone exists as vapor. Due to the atmospheric half-life, which is on the order of days (see Section 5.3.2.1), acetone will be transported long distances in the air. Although not a large sink (Chatfield et al. 1987), small amounts of acetone will be removed from the atmosphere by wet deposition (Grosjean and Wright 1983), which will transport acetone from the atmosphere to surface water and soil.

The complete miscibility of acetone in water suggests that partitioning of acetone from the water column to sediments and suspended solids in water is not significant. The estimated low value of 0.73 for log K_{oc} (see Table 3-2) also suggests that adsorption of acetone to sediments and suspended solids is not significant. In the absence of water, acetone vapor adsorbs rather strongly to the clay component of soil by hydrogen bonding (Goss 1992; Steinberg and Kreamer 1993). The sorption is

dependent on relative humidity, and increasing the humidity decreases sorption drastically. In water saturated soil or sediment, only organic carbon, as indicated by K_{oc}, and not hydrogen bonding may control the sorption of acetone (Steinberg and Kreamer 1992). The experimental adsorption studies with kaolinite, monkmorillonite, and stream sediments showed very little or no loss of acetone from water to the adsorbents (Rathbun et al. 1982). The transport of acetone from the water column to the atmosphere depends on the Henry's law constant. The Henry's law constant for acetone is $4.26x10^{-5}$ atm-m^3/mol (see Table 3-2). Therefore, volatilization of acetone from water, although not very fast, is significant (Thomas 1982). The volatilization rate of a chemical depends on the characteristics of the chemical and the water and on other ambient conditions (e.g., water depth, suspended solid concentration, water current, wind speed, temperature). Based on an estimation method (Thomas 1982) and a Henry's law constant value of $4.26x10^{-5}$ atm-m^3/mol, the volatilization half-life of acetone from a model river 1 m deep, flowing at a current of 1 m/second with a wind velocity of 3 m/second is about 18 hours. The mean volatilization coefficient for acetone in a model outdoor stream was in the range of $7.15x10^{-4}$ to $14.8x10^{-4}$/minute (Rathbun et al. 1989, 1991). Therefore, the volatilization half-life of acetone from the model stream is in the range of 7.8-16.2 hours. It was concluded that volatilization will control the fate of acetone in water (Rathbun et al. 1989, 1991). Results of a laboratory study (Rathbun et al. 1982) also concluded that volatilization. is one of the important fate determining processes for acetone in streams.

The log K_{ow} value of -0.24 (see Table 3-2) suggests that bioconcentration of acetone in aquatic organisms is not significant. The measured bioconcentration factor for acetone in adult haddock exposed to acetone under static conditions at 7-9°C was <l (Rustung et al. 1931). No data regarding the biomagnification potential of acetone in aquatic organisms were located; however, the low K_{ow} value suggests that biomagnification of acetone from animals of lower to higher trophic level is unlikely.

The two significant transport properties for acetone in soil are volatilization and leaching. Leaching transports acetone from soil to groundwater. The rate of leaching from soil by rainwater depends on the sorption characteristics of acetone in soil. Since acetone may be controlled by K_{oc} in water-saturated soil and has a low K_{oc} value, sorption of acetone in such soil will be weak. The low retention ability will permit acetone to leach into groundwater. A sorption study with moist clay soils indicates that aqueous acetone causes swelling in these soils (Green et al. 1983), and this process may allow the retention of a small fraction of acetone. Groundwater monitoring studies (see Section 5.4.2)

at landfill sites provided evidence of the importance of acetone leaching from soil. Volatilization transports acetone from soil to the atmosphere. The volatility rate of acetone from soil depends on the soil characteristics (moisture content, soil porosity, etc.). Since the acetone is weakly sorbed to soil, the volatility depends primarily on the moisture content of the soil. In dry soil, the volatilization rate from soil surfaces is high due to the high vapor pressure of acetone. In moist soil, the rate of volatilization is similar to acetone in water and depends on the Henry's law constant. Acetone volatilizes moderately under these conditions. The detection of acetone at higher concentrations in downwind air of a landfill site, compared to upwind air (Militana and Mauch 1989), indicates the importance of volatilization as a transport process in soil.

No data regarding the transport of acetone from soil to plants were located.

5.3.2 Transformation and Degradation

5.3.2.1 Air

The reactions of acetone vapor with nitrogen oxides, hydroxyl radicals (OH), singlet molecular oxygen ($^1\Delta_g$, singlet atomic oxygen (O[^{3}P]), and nitrate radicals have been studied. Given the second order rate constants for the reactions of acetone with $^1\Delta_g$ (Datta and Rao 1979) and O(^{3}P) (Lee and Timmons 1977), and the concentrations of singlet molecular and atomic oxygen in the atmosphere (Graedel 1978), these reactions are insignificant in determining the fate of acetone in the atmosphere. The reaction of acetone with nitrate radicals in the atmosphere was also determined to be insignificant (Boyd et al. 1991). Smog chamber studies with acetone and nitrogen oxides conclude that acetone has low reactivity in terms of ozone and nitrogen dioxide formation and that the rate of disappearance of acetone by this process is low (Altshuller and Cohen 1963; Dimitriades and Joshi 1977; Yanagihara et al. 1977). The photochemical oxidation of acetone in the presence of nitrogen oxides produces small amounts of peroxyacetic acid and peroxyacetyl nitrate (Hanst and Gay 1983).

The two significant processes in determining the fate of acetone in the atmosphere are reaction with hydroxyl radicals and photolysis. The rate constant for the reaction of hydroxyl radicals with acetone at 25°C is in the range of 2.2-5.0x10^{-13} cm^3/molecule-second (Cox et al. 1980; Cox et al. 1981; Meyrahn et al. 1986). The estimated average lifetime of acetone due to reaction with hydroxyl radicals is 44.5 days (Meyrahn et al. 1986). The probable pathways for the reaction of acetone with

hydroxyl radicals in the troposphere have been postulated, and methylglyoxal is the primary product of this reaction (Altshuller 1991). Acetone underwent significant photolysis with an artificial light (cutoff wavelength of 270 nm) with a maximum emission intensity of 300 nm (Fujiki et al. 1978). Besides free radicals, the primary products of acetone photolysis in sunlight are carbon dioxide and acetylperoxynitrate (Altshuller 1991). The lifetimes of acetone due to photolysis under cloudless conditions at 40º N latitude, and at sea level during winter and summer are estimated to be 83 and 19 days, respectively (Martinez et al. 1992). Other investigators have estimated that the average atmospheric lifetime of acetone due to photolysis at 40º N latitude is 115.7 days/year (Meyrahn et al. 1986). The estimated average lifetime of acetone at 40º N due to combined hydroxyl radical reaction and photolysis is 32 days/year (Meyrahn et al. 1986), corresponding to a half-life of 22 days. Due to the pressure dependence of the quantum yield, the rate of photodissociation will increase as altitude increases, whereas the reaction rate with hydroxyl radicals will decrease because temperature decreases at higher altitudes. Therefore, the lifetime of acetone in the atmosphere will remain approximately constant with respect to altitude. However, the rate will show a pronounced dependence on latitude with greater losses of acetone occurring near the equator, compared to the poles (Meyrahn et al. 1986).

5.3.2.2 Water

Based on the rate constant for the reaction of acetone with hydroxyl radicals in water at pH 7 ($5.8\text{-}7.7 \times 10^{7}$/M-second) (Anbar and Neta 1967) and the concentration of hydroxyl radicals in eutrophic waters (3×10^{-17}M) (Mill and Mabey 1985), this reaction will not be significant in water. When distilled water or natural water containing acetone were exposed to sunlight for 2-3 days, no photodecomposition of acetone was observed (Rathbun et al. 1982). Therefore, photolysis of acetone in water is not an important process.

Many aerobic biodegradation screening studies with mixed microorganisms from waste-treatment plant effluents, activated sludge, or sewage have examined the biodegradability of acetone (Babeu and Vaishnav 1987; Bhattacharya et al. 1990; Bridie et al. 1979; Ettinger 1956; Gaudy et al. 1963; Hatfield 1957; Heukelekian and Rand 1955; Lamb and Jenkins 1952; Price et al. 1974; Stafford and Northup 1955; Thorn and Agg 1975; Urano and Kato 1986a, 1986b). These studies indicate that acetone is easily biodegradable with acclimatized microorganisms or after a suitable lag period (≈ l day) (Urano and Kato 1986a, 1986b), as long as the initial concentration of acetone is not at a toxic level. For example, acetone at a concentration of 500 mg/L was toxic to microorganisms when biooxidation of

acetone by activated sludge was attempted (Gerhold and Malaney 1966). Biodegradation of acetone was much slower in seawater than in fresh water (Takemoto et al. 1981). After a suitable lag period (5 days), acetone biodegraded quantitatively under anaerobic conditions with anaerobic acetate enriched culture medium (Chou et al. 1979). A biodegradation study of acetone in natural water collected from Lago Lake near Athens, Georgia, determined that the biodegradation kinetics is multiphasic in nature and depends on the substrate concentration. The determined rate of degradation was faster at higher initial concentrations (the maximum concentration used was 0.5 mg/L) (Hwang et al. 1989).

In a laboratory experiment with natural stream water and sediment, no acetone was lost in 338 hours under sterile conditions in closed flasks. However, with nonsterile natural sediment, 100% of the acetone was lost in 500 hours following a lag period of 90 hours. (Rathbun et al. 1982). The authors of this study concluded that biodegradation was one of the important processes for the loss of acetone in streams. Significant loss of acetone due to biodegradation was not observed in a later study where acetone was injected continuously in an outdoor model stream (Rathbun et al. 1988, 1989, 1991, 1993). Attempts to induce biodegradation by adding glucose and a nutrient solution containing bacteria acclimated to acetone were unsuccessful. The authors concluded that the residence time of acetone in the model stream (6 hours) was too short for the bacteria to become acclimated in the water before initiation of biodegradation. However, this explanation may not be valid if attached bacteria, rather than free-floating bacteria, dominate the biodegradation process. As an alternative explanation, the authors indicated that the observed limitation in the nitrate concentration in the stream may be responsible for the lack of acetone biodegradation.

5.3.2.3 Sediment and Soil

The biodegradation studies discussed in Section 5.3.2.2 indicate that biodegradation of acetone in sediment and soil will be significant. However, laboratory or field data examining the biodegradability of acetone in soil are lacking. No evidence was located to suggest that any degradation process other than biodegradation is important in sediment and soil.

5.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

5.4.1 Air

Acetone is a volatile compound and is stable in air. Therefore, some monitoring data for the levels of acetone in air are available. The levels of acetone in urban, rural, and remote areas in the United States and the level in the troposphere are shown in Table 5-2.

It is obvious from Table 5-2 that the concentration of acetone in the air around urban areas will be higher than rural and remote areas because of higher emission rates from a larger number of sources. Besides these data, air monitoring data from an urban area (Tulsa, Oklahoma), a rural area (Rio Blanco County, Colorado), and a remote area (Smoky Mountain, Tennessee) are also available (Arnts and Meeks 1981). These data are not presented in Table 5-2 because the samples were collected in Tedlar bags that are known to contaminate air samples with acetone. As a result, the reported acetone concentrations were consistently higher than the values given in Table 5-2. Table 5-2 also indicates that the indoor concentration of acetone is generally higher than the outdoor concentration. Other investigators reported similar results (Jarke et al. 1981). The reason for the higher indoor air concentration is the use of acetone-containing consumer products inside homes. The potential for intrusion of acetone present as soil gas into a house adjacent to a landfill by diffusive and advective routes was found to be low (Hodgson et al. 1992). However, only a single house was studied, and the ambient air in the basement of the house was estimated on two separate occasions and found to be 12 and 82 ppb (v/v).

5.4.2 Water

In a National Organics Reconnaissance Survey (NORS) by EPA involving drinking water supplies from 10 cities in the United States, acetone was qualitatively detected in all 10 water samples. The 10 cities in this survey were Cincinnati, Ohio, Miami, Florida, Ottumwa, Indiana, Philadelphia, Pennsylvania, Seattle, Washington, Grand Forks, North Dakota, Lawrence, Kansas, New York, New York, Terrebonne Parrish, Louisiana, and Tucson, Arizona (Bedding et al. 1982; Coleman et al. 1976; Keith et al. 1976). The determined concentration of acetone in one of the drinking water samples (Seattle, Washington) was 1 ppb (Keith et al. 1976). Acetone has also been detected in water from several artesian wells adjacent to a landfill in Wilmington, Delaware and at a concentration of 0.3 ppb

TABLE 5-2. Acetone Levels in the Troposphere and Urban, Rural, and Remote Areas in the United States and Canada

Location	Monitoring year	Concentration ppb (v/v)	Reference
Tucson, AZ	1982	12.0	Snider and Dawson 1985
Houston, TX	1987–1988	6.1	Lagrone 1991
U.S. outdoor	1980–1986	6.9	Shah and Singh 1988
U.S. indoor	1980–1986	8.0	Shah and Singh 1988
Indoor air of a newly constructed office building, Portland, OR	1987–1988	12.1–28.1	Hodgson et al. 1991
Rural, AZ	1982	2.8	Snider and Dawson 1985
Rural, Ontario	1988	1.7	Shepson et al. 1991
Background, clean air	1988	0.38	Zhou and Mopper 1993
Point Barrow, AK	1967	1.1	Cavanagh et al. 1969
Troposphere (lower)	—	0.7	Dilling 1982
Troposphere (lower)	1984–1985	0.47	Arnold et al. 1986
Troposphere (upper)	1984–1985	0.12	Arnold et al. 1986

in finished drinking water from one of the wells (DeWalle and Chian 1981). The concentration of acetone was ≤3,000 ppb in a drinking water well in New Jersey (Burmaster 1982; Steelman and Ecker 1984).

The concentration of acetone in open ocean water (Tongue of the Ocean, Bahamas) was 6 nM (0.35 ppb) (Kieber and Mopper 1990) whereas the reported mean concentrations in seawater from Straits of Florida and the Eastern Mediterranean were 20 and 30 ppb, respectively (Corwin 1969). The concentration of acetone in the Potomac River, VA was below the detection limit of 40 ppb (Hall et al. 1987). Acetone has been detected in the effluent from a textile plant (Gordon and Gordon 1981) and in effluent water from a specialty chemicals manufacturing plant at a concentration of 200-230 ppm (Jungclaus et al. 1978). The compound has also been detected in groundwater, leachate, and run-off waters from landfill sites (Brown and Donnelly 1988; DeWalle and Chian 1981; Gould et al. 1983; Sawhney and Raabe 1986; Stonebraker and Smith 1980). The concentration of acetone in an industrial landfill leachate in Michigan was in the range of 0.05 to 62.0 ppm (Brown and Donnelly 1988). However, the quality of the reported data is uncertain. Acetone was detected at a mean concentration of 56 ppb in a landfill leachate in Orange County, Florida (Hallbourg et al. 1992).

5.4.3 Sediment and Soil

There are few data regarding the level of acetone in soil and sediment. Acetone has been detected in 43% of the soil samples in Superfund sites for which acetone determination has been made so far. The maximum concentration of acetone in soils from Vega Alta Public Supply well sites in Puerto Rico was 9,500 ppb (ATSDR 1988). The mean concentration of acetone in soil from Summit National Site, Ohio, was 9,484 ppb (dry weight) (EPA 1988b). Acetone has been qualitatively detected in river sediment that received effluents from a specialty chemicals manufacturing plant (Hites and Lopez-Avila 1980). A concentration of 6 ppb μg/kg) acetone was detected in the sediment of a creek adjacent to a landfill in Louisville, Kentucky (Stonebraker and Smith 1980). Because of its high water solubility and low sediment adsorption coefficient, most acetone in an aquatic system will be found in water, rather than in sediment.

5.4.4 Other Environmental Media

Acetone has been qualitatively detected as a volatile component of a number of foods including blue cheese (Day and Anderson 1965), baked potatoes (Coleman et al. 1981), roasted filbert nuts (Kinlin et al. 1972), meat (Grey and Shrimpton 1967; Shahidi et al. 1986), and nectarines (Takeoka et al. 1988). In a study carried out in Czechoslovakia, the concentrations of acetone in samples of milk and cream culture were 79.5 and 0.11 mg/l00 kg, respectively (Palo and Ilkova 1970). Acetone also has been qualitatively detected in breast milk of working mothers, although the study did not identify whether the concentrations of acetone were higher than normal physiologic levels (Giroux et al. 1992). Acetone has been qualitatively detected in 8 of 12 mothers' milk samples collected from two locations in New Jersey, Bridgeville, Pennsylvania, and Baton Rouge, Louisiana (Pellizzari et al. 1982). In kiwi fruit, the acetone concentration comprised 0.2% of total volatile components (Bartley and Schwade 1989). The concentrations of acetone in dry legumes, such as beans (mean of several varieties), split peas, and lentils were 880, 530, and 230 ppb, respectively (Lovegren et al. 1979). The level of acetone in headspace volatiles of Bisbee Delicious apples ranged from 111 to 912 pL/kg-hour (Mattheis et al. 1991). The percent of acetone (of the total) in commercial concentrated aqueous orange essences ranged from 0.003 to 0.009 (Moshonas and Shaw 1990).

Acetone has been detected in occasional rain samples collected in Hanover, Germany (Levsen et al. 1990). The authors were not sure whether the detection of acetone in the rain water was due to contamination of samples during analysis. The concentration of combined acetone and acrolein was 0.05 ppm in a rain water sample from Los Angeles, California (Grosjean and Wright 1983). The investigators could not separate acetone from acrolein by the method used for the determination of carbonyl compounds.

5.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

Acetone is endogenously produced by all humans. The general population is exposed to acetone by inhaling ambient air, ingesting food, and drinking water containing acetone. Dermal exposure to acetone may result from skin contact with certain consumer products (e.g., certain nail polish removers, paint removers, and household cleaning and waxing products) (see Section 5.2.1). However, no quantitative data for dermal exposure to acetone from consumer products were located. Assuming concentrations of acetone are 8.0 ppb (18.99 $\mu g/m^3$) in indoor air and 6.9 ppb (16.38 ug/m^3) in outdoor

air (Shah and Singh 1988) and that a person inhales 15 m^3/day of indoor air and 5 m^3/day of outdoor air, the estimated inhalation rate of acetone is 0.37 mg/day. No experimental or estimated data were located regarding the daily intake of acetone in the general population in the United States from ingestion of drinking water and food. However, if the concentration of acetone in drinking water is $\leq$1 ppb (see Section 5.4.2), the daily intake for acetone (assuming a person consumes 2 L of drinking water/day) from this source would be negligible.

The acetone concentrations in body fluids and expired air of healthy and diabetic patients are given in Table 5-3. The concentration of acetone in whole blood does not differ from that in plasma (Gavin0 et al. 1986). Even in healthy subjects, the level of acetone in blood/plasma varies with fasting or nonfasting conditions and depends on the weight of the subject. Generally, the blood/plasma acetone concentrations are higher in fasted than nonfasted subjects and higher in subjects who are not obese, compared to obese subjects (Haff and Reichard 1977).

Workers in industries that manufacture and use acetone can be exposed to much higher concentrations of acetone than the general population. For example, the concentrations of acetone in the breathing zone air in a paint factory, a plastics factory, and an artificial fiber factory in Italy were >3.48 mg/m^3 (Pezzagno et al. 1986). The concentration of acetone in the breathing zone air of a fiber-reinforced plastic plant in Japan, where bathtubs were produced, was $\leq$108 mg/m^3 (Kawai et al. 1990a). The inhalation exposure for workers to acetone in a shoe factory in Finland ranged from 25.4 to 393.4 mg/m^3 (Ahonen and Schimberg 1988). Concentrations of acetone in the breathing zone air in shoe factories in Italy were also high (Brugnone et al. 1978). The concentration of acetone in the breathing zone air of a solvent recycling plant in the United States ranged from not detected to 43 mg/m^3 (Kupferachmid and Perkin 1986). High levels of acetone were detected in the occupational air in other industries including chemical, plastic button, and paint manufacturing industries in Italy (Ghittori et al. 1987). Isopropyl alcohol is known to oxidize in the liver and is converted to acetone (Kawai et al. 1990b). Therefore, occupational exposure (printing plants) or accidental ingestion of isopropyl alcohol also produce acetone in expired air, blood, and urine. The National Occupational Exposure Survey (NOES) statistically estimated that 1.51 million workers were potentially exposed to acetone in the United States in 1980 (NIOSH 1989). The NOES database does not contain data on the frequency, duration, concentration, or route of exposure of workers to chemicals.

TABLE 5-3. Concentrations of Acetone in Body Fluids and Expired Air of Humans

Subject	Medium	Concentration	Reference
Healthy (nonfasted)	Blood	0.016 mM (0.93 mg/L)	Gavino et al. 1986
Healthy (fasted)	Plasma	0.8 mM (46.5 mg/L)	Haff and Reichard 1977
Healthy (nonfasted)	Plasma	0.03 mM (1.74 mg/L)	Trotter et al. 1971
Ketoacidotic	Plasma	7.3 mM (424 mg/L)	Trotter et al. 1971
Ketoacidotic	Plasma	5.0 mM (290 mg/L)	Haff and Reichard 1977
Healthy	Urine	4–7 μM (0.23–0.41 mg/L)	Kobayashi et al. 1983
Healthy (endogeneous)	Urine	13.1 μM (0.76 mg/L)	Pezzagno et al. 1986
Diabetic	Urine	11–155 μM (0.64–9.0 mg/L)	Kobayashi et al. 1983
Healthy	Expired air	0.52 ppm (1.23 μg/L)	Jansson and Larsson 1969
Healthy	Expired air	0.02 μM (1.16 μg/L)	Trotter et al. 1971
Healthy	Expired air	0.023 μM (1.3 μg/L)	Phillips and Greenberg 1987
Healthy	Expired air	101.3 ng/L[a] (0.1 μg/L)	Krotoszynski et al. 1979

[a]The reason for the lower value reported by these investigators is not known with certainty but may be due to different sampling procedures (e.g., Teflon® versus polyester sampling bags).

5.6 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Workers in industries that manufacture or use acetone are one segment of the population at an especially high risk of acetone exposure compared to the general population (see Section 5.5). Professional painters and commercial and household cleaners (certain detergents, cleansers, waxes, or polishes contain acetone) are also likely to be exposed to acetone at higher concentrations than the general population, although experimental data regarding the extent of exposures for this segment of workers were not located. Among the general population, high exposure to acetone may occur among several subgroups. Cigarette smoke contains ≤0.54 mg acetone/cigarette (Manning et al. 1983); therefore, smokers are exposed to higher concentrations of acetone than nonsmokers. The content of acetone in certain nail polish removers is high; therefore, individuals who frequently use nail polish removers are exposed to higher levels of acetone than the general population. People who live near landfill sites that emit acetone or those who live near industrial sources of emission (e.g., refinery, incinerator, close to high vehicular traffic areas) are also susceptible to higher exposure concentrations of acetone than the general population that does not reside near these sites. People who consume contaminated well water (see Section 5.4.2) as drinking water are subject to high exposures. People who consume food containing acetone excessively would also be subject to high exposure, especially if associated with other risks.

5.7 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of acetone is available. Where adequate information is not available, ATSDR, in conjunction with the NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of acetone.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

5.7.1 Identification of Data Needs

Physical and Chemical Properties. Information regarding the physical and chemical properties of acetone necessary to predict its environmental fate and transport processes in the environment is available (see Table 3-2). However, experimental determination of a value for the soil sorption coefficient of acetone from water would be helpful in assessing the potential for leaching and volatility of acetone in different soils.

Production, Import/Export, Use, Release, and Disposal. Data regarding the past and present production volume for acetone and the projected future trend (increase/decrease) in the production volume are known (CMR 1990). In 1990, the reported total production volume of acetone in the United States was 2,330 million pounds (USITC 1991). Recent export and import data for acetone would be helpful. The use pattern of acetone is known. Acetone is present in several household consumer products, but by far a larger amount of acetone is used in industry. Acetone is naturally present in many fruits and vegetables, and there is no information available to indicate that acetone is present in food as a contaminant, due to food processing or packaging. Air is the most likely environmental medium in which significant quantities of acetone contamination will occur during its production and use (TRI90 1992). More data on the rate of acetone release from household consumer products would be useful. The regulations governing the disposal of acetone are well defined. However, more information about the proportion of discarded acetone recovered from recycling, and the proportion lost due to evaporation, ground burial, and incineration would be useful in determining the relative importance of the different routes of exposure.

According to the Emergency Planning and Community Right-to-Know Act of 1986, 42 U.S.C. Section 11023, industries are required to submit chemical release and off-site transfer information to the EPA. The Toxics Release Inventory (TRI), which contains this information for 1988, became available in May of 1990. This database will be updated yearly and should provide a list of industrial production facilities and emissions.

Environmental Fate. The environmental fate of acetone, for the most part, has been well studied (see Section 5.3). Acetone will undergo transport from one environmental medium to another (Grosjean and Wright 1983; Rathbun et al. 1982). Due to its reasonably long half-life in air (22 days) (Meyrahn et al. 1986) and restricted volatilization from groundwater, the atmosphere and groundwater

may act as sinks for acetone. More experimental data regarding the rate of sorption and biodegradation of acetone in soil and its biodegradability in groundwater would be useful to assess the relative importance of the different fate processes.

Bioavailability from Environmental Media. Acetone is readily absorbed in the lung and gastrointestinal tract following inhalation and ingestion. Acetone can also be absorbed from the skin (see Section 2.3). The low value for K_{oc} (see Table 3-2) and a moderate value for Henry's law constant (Rathbun and Tai 1987) suggest that bioavailability of acetone from contaminated water and soil as a result of skin contact may be significant. However, quantitative data regarding the rate and extent of dermal absorption of acetone from contaminated water and soil are lacking. The high water solubility and low K_{oc} value for acetone suggest that bioavailability from ingested soil (e.g., children playing at or near contaminated sites) will be high, but quantitative absorption data are lacking. Data on bioavailability of acetone from ingested plant food were not located but would be helpful.

Food Chain Bioaccumulation. Acetone does not bioaccumulate in aquatic organisms. There is no indication of biomagnification of acetone along the aquatic food chain. Studies indicating the potential for acetone transfer from soil and plants and biomagnification in terrestrial food chains would be useful to confirm its potential for food chain bioaccumulation.

Exposure Levels in Environmental Media. Data regarding the level of acetone in ambient air are available (Lagrone 1991; Shah and Singh 1988; Snider and Dawson 1985). There is a paucity of data regarding the level of acetone in drinking water (Bedding et al. 1982; Coleman et al. 1976; Keith et al. 1976). More comprehensive data on the levels of acetone in the air and water consumed by people who live near acetone-containing hazardous waste sites would be useful in estimating the daily intake from these sources. Although the levels of acetone in the volatile components of several fruits and vegetables are available (see Section 5.4.4), development of data regarding the level of acetone in the total diet would be useful. There are few data regarding the level of acetone in background soil samples.

Reliable monitoring data for the levels of acetone in contaminated media at hazardous waste sites are needed so that the information obtained on levels of acetone in the environment can be used in combination with the known body burden of acetone to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

Exposure Levels in Humans. The levels of acetone in blood/plasma and urine of healthy people, occupationally exposed groups, and diabetic patients are available (see Table 5-3 and Section 5.5). However, data on the levels of acetone in body fluids or tissues of general populations living near sites with higher (than normal) exposure potential (e.g., hazardous waste sites) were not located. This information is useful for assessing the need to conduct health studies on these populations.

Exposure Registries. No exposure registries for acetone were located. This substance is not currently one of the compounds for which a subregistry has been established in the National Exposure Registry. The substance will be considered in the future when chemical selection is made for subregistries to be established. The information that is amassed in the National Exposure Registry facilitates the epidemiological research needed to assess adverse health outcomes that may be related to exposure to this substance.

5.7.2 On-going Studies

No on-going study that would fill the data gaps regarding the transport and fate of acetone in the environment or that evaluates its exposure potential in general population groups susceptible to higher levels of exposure was located.

As part of the Third National Health and Nutrition Evaluation Survey, the Environmental Health Laboratory Sciences Division of the National Center for Environmental Health and Injury Control, Centers for Disease Control, will be analyzing human blood samples for acetone and other volatile organic compounds. These data will give an indication of the frequency of occurrence and background levels of these compounds in the general population.

6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, and/or measuring, and/or monitoring acetone, its metabolites, and other biomarkers of exposure and effect to acetone. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

6.1 BIOLOGICAL MATERIALS

Some of the more recent methods for determining the presence of acetone in different biological media are reported in Table 6-1. Prior to the 1960s acetone was considered to be present in only insignificant amounts in hyperketonic states in healthy people. Therefore, the concentration of acetone in biological fluids was rarely measured. The effort to develop analytical methods for the determination of acetone in body fluids increased when it was found that the level of acetone in diabetic patients with severe hyperketonaemia may indeed be significant (Trotter et al. 1971). The parent acetone or its reduction/derivatization product is almost exclusively determined by GC/FID method, although derivatized products can be determined by high-performance liquid chromatography with ultraviolet or fluorescence detectors (Brega et al. 1991; Vairavamurthy et al. 1992). Since the reference acetone values for healthy unexposed people are 0.034-0.120 mmol/L, with a mean of 0.075 mmol/L in plasma, and 0.034-0.095 mmol/L, with a mean value of 0.052 mmol/L in urine (Brega et al. 1991), the available methods have adequate sensitivity to determine the levels of acetone in biological samples of both healthy and diabetic people (Gavin0 et al. 1986; Kobayashi et al. 1983; Phillips and Greenberg 1987; Trotter et al. 1971). The determination of acetone in blood is difficult because acetone is generated as a metabolite from acetoacetate (major metabolite is βhydroxybutyrate), and the quantity produced depends on storage time even when the blood samples are stored at 4°C (Trotter et al. 1971). In addition, acetylacetone present in the plasma is thermally degraded to

TABLE 6-1. Analytical Methods for Determining Acetone in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Whole blood	Sample centrifuged to obtain plasma; plasma deproteinized with $HClO_4$	GC-FID	6.6 ng/mL (0.023 nmol)	No data	Preinado et al. 1987
Whole blood	Deproteinized with $HClO_4$ and subjected to purge-and-trap	GC-FID	23.2 ng/mL (0.4 μM)	90–92%	Mangani and Ninfali 1988
Whole blood	Purge-and-trap	GC-MS	0.2 μg/mL	87–107%	Ashley et al. 1992
Serum	Deproteinized with sodium tungstate and cupric sulfate	HRGC-FID	<58 μg/mL (<1 mmol/L)	No data	Smith 1984
Plasma	Acetylacetone in sample enzymatically converted to ß-hydroxybutyrate and sample distilled with H_2SO_4	Automated coulometric with salicylaldehyde in KOH	1.16 μg/mL (0.02 μmol/mL)	95%	Haff and Reichard 1977
Serum	Sample centrifuged and clear filtrate injected into GC	GC-FID	5.8 ng/mL (0.1 nmol/mL)	No data	Cheung and Lin 1987
Urine	Diluted sample derivatized with pentafluorobenzyloxylammonium chloride and extracted with hexane	GC-FID	0.2 μg/mL (3.45 μmol/mL	No data	Kobayashi et al. 1983
Liver	Liver perfusion medium reduced with $NaBH_4$ and an aliquot of reduced solution injected into GC	GC-FID	3.78 μg/mL in perfusate (65 μM)	No data	Holm and Lundgren 1984

TABLE 6-1. Analytical Methods for Determining Acetone in Biological Materials (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Liver, kidney, lung, and adipose tissue	Purge-and-trap	GC-FID	No data	52% (lung) 98% (other tissue)	Holm and Lundgren 1984
Breath	Direct injection into GC	GC-FID	No data	98%	Trotter et al. 1971; Jansson and Larsson 1969

H_2SO_4 = sulfuric acid; FID = flame ionization data; GC = gas chromatography; $HClO_4$ = perchloric acid; HRGC = high resolution gas chromatography; KOH = Potassium hydroxide; MS = mass spectrometry; $NaBH_4$ = sodium borohydride

acetone inside the injection port and on the column, if a gas chromatographic method is used for quantification (Gavino et al. 1986; Trotter et al. 1971).

Two methods that have been used to eliminate the interference from acetylacetone are enzymatic conversion of acetoacetate to 3-hydroxybutyrate and chemical reduction of acetone to 2-propyl alcohol (Gavin0 et al. 1986; Haff and Richard 1977). Another factor that makes determination of acetone unreliable is the increased evaporative loss of acetone from the samples as the time of storage is prolonged (Trotter et al. 1971). Acetone was detected in several brands of heparin (Powell et al. 1985). Therefore, determination of acetone in heparinized blood may give erroneous results if the heparin is contaminated with acetone.

6.2 ENVIRONMENTAL SAMPLES

Some of the analytical methods for determining acetone in environmental samples are reported in Table 6-2. In addition to these methods, personal sampling methods have been developed in Japan for the determination of acetone in the workplaces (Uchida et al. 1990). There is no unique method that can be used for the determination of low levels of acetone in unpolluted environmental samples. As in the case of biological samples, the sensitivity of acetone detection in environmental samples can be enhanced by derivatization. Such a method, which uses 2,4-dinitrophenylhydrazine as a derivatizing agent, has been applied for the determination of extremely low levels of acetone in open ocean sea water (Kieber and Mopper 1990). A new method based on the reaction of mercuric oxide with carbonyl compounds has been applied for the determination of low levels of acetone in unpolluted atmosphere (O'Hara and Singh 1988). A good review of the methods presently used for the determination of acetone in the atmosphere is available (Vairavamurthy et al. 1992). Unlike in biological samples, acetylacetone may not be present in most environmental samples. If acetylacetone is detected in an environmental sample, thermal degradation inside the gas chromatographic system (injection port and column oven) may produce erroneous results unless this interference is eliminated. As in the case of biological samples, proper precautions in sample handling during collection and analysis, and storage of environmental samples should be taken to minimize contamination and evaporative loss. It has been suggested that ambient water containing low levels of acetone be sampled upwind of sources of contamination (e.g., smoke stack or other sources of emissions) and that the operator stand downwind of the samples while withdrawing them (to minimize contamination from operator's breath) (Kieber and Mopper 1990).

TABLE 6-2. Analytical Methods for Determining Acetone in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air (occupational)	Air passed through charcoal and components disorbed with CS_2	GC-FID (NIOSH method 1300)	0.007 mg/L	No data	NIOSH 1990
Air (occupational)	Air passed through Porapak N and components thermally desorbed	GC-FID	<0.01 ppm (v/v)	110% at 0.52 ppm	Campbell and Moore 1977
Air (occupational)	Air passed through charcoal and components desorbed with CS_2	GC-FID	<0.05 mg/L	74–92%	Otson et al. 1983
Air (ambient)	Air passed through a cryogenic trap and the trapped component injected into GC	GC-RGD	10 ppt	No data	O'Hara and Singh 1988
Air	Air passed through a 1% sodium bisulfite solution and absorbed acetone reacted with alkaline vanilla solution	Spectrophotometry	<0.5 ppm (in solution)	No data	Amlathe and Gupta 1990
Rural air	Air passed through silica gel coated with 2,4-dinitrophenyl-hydrazine and eluted with acetonitrile	HPLC-UV	No data	<90%	Shepson et al. 1991
Water	Sample reacted with alkaline diazotized anthranilic acid solution	Spectrophotometry	0.5 mg/L	98.5–102%	Rahim and Basir 1981

TABLE 6-2. Analytical Methods for Determining Acetone in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Sample reacted with sodium bisulfite	IC/conductivity detection	No data	No data	DuVal et al. 1985
Fresh and seawater	Sample derivatized with 2,4-dinitrophenylhydrazine passed through a C_{18} cartridge and adsorbed compound eluted with acetonitrile	HPLC-UV detection	0.5 nM (0.03 μg/L)	No data	Kieber and Mopper 1990
Waste water, soil or sediment	Sample or sample mixed with reagent water subjected to purge-and-trap	GC-MS (EPA method 8240)	100 μg/L (water) 100 μg/kg (sediment and soil)	No data	EPA 1986
Fresh fruit	Vacuum distillation followed by solvent extraction of pulp	HRGC-MS	No data	No data	Takeoka et al. 1988

CS_2 = carbon disulfide; FID = flame ionization detector; GC = gas chromatography; HPLC = high performance liquid chromatography; HRGC = high resolution gas chromatography; IC = ion chromatography; MS = mass spectrometry; RGD = reduction gas detector; UV = ultraviolet; v/v = volume per volume

6.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of acetone is available. Where adequate information is not available, ATSDR, in conjunction with the NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of acetone.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. The concentration of acetone in expired air, blood, and urine can serve as indicators of recent exposure to acetone (Ghittori et al. 1987; Pezzagno et al. 1986; also see Section 2.5.1.). Concentrations of acetone similar to those produced from occupational exposure are also produced in blood and urine as a result of inhalation and ingestion of isopropyl alcohol (may be present as a contaminant in some alcoholic beverages) (Kawai et al. 1990b) and in patients with diabetes mellitus (Dobson et al. 1968; Trotter et al. 1971). Among urine, alveolar air, and blood, the strongest correlation was found between the time-weighted average breathing zone air concentration and urinary acetone level when the concentration of acetone in breathing zone air exceeded 15 ppm (v/v) (Fujino et al. 1992; Kawai et al. 1992). Analytical methods of adequate sensitivities are available for determining acetone in blood, urine, and expired air at background levels in the population and levels at which biological effects occur (Gavin0 et al. 1986; Kobayshi et al. 1983; Phillips and Greenberg 1987; Trotter et al. 1971). Although the precision and accuracy of the analytical methods have not always been reported (e.g., Robayashi et al. 1983), these values are satisfactory in cases where they have been reported (Haff and Reichard 1977; Holm and Lundgren 1984; Mangani and Ninfali 1988; Trotter et al. 1971).

Biological monitoring has not been used to associate acetone exposure levels with specific effects that can be quantified in humans (see Section 2.5.2). Clinical tests for nonspecific effects of acetone exposure (see Section 2.9.2) are available (Johansson et al. 1988; Lupulescu et al. 1972, 1973; Tosti et al. 1988). Since acetone exposure itself can be quantified in biological tissues, there does not appear to be a need to improve existing clinical tests or develop additional test procedures to detect effects of acetone exposure.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods for determining low levels of acetone found in ambient air are available (Ants and Meeks 1981; Cavanagh et al. 1969; O'Hara and Singh 1988; Shepson et al. 1991; Snider and Dawson 1985). Methods are also available for determining low levels of acetone found in natural waters (Kieber and Mopper 1990). The concentrations of acetone found in volatile components of several foods have also been determined (Bartley and Schwede 1989; Day and Anderson 1965; Takeoka et al. 1988). The degradation products formed as a result of photochemical reactions of acetone in air in the presence or absence of nitrogen oxides have been identified (Altshuller and Bufalini 1971; Boule et al. 1987; Hanst and Gay 1983; Meyrahn et al. 1986). However, products from the reaction of acetone with hydroxyl radicals and $O(^3P)$ remain unidentified (Cox et al. 1980; Lee and Timmons 1977), although it has been postulated that methylglyoxal may be the principal product of the reaction with hydroxyl radicals (Altshuller 1991). It would be helpful to determine the products formed from the gas phase reaction of acetone with hydroxyl radicals in order to assess the potential health effect implications of these products.

6.3.2 On-going Studies

No on-going studies that could free the data gap regarding the products formed from the reactions of acetone with atmospheric oxidants (e.g., hydroxyl radicals, $O[^3P]$) were found.

The Environmental Health Laboratory Sciences Division of the National Center for Environmental Health and Injury Control, Centers for Disease Control, is developing methods for the analysis of acetone and other volatile organic compounds in blood. These methods use purge-and-trap methodology, high resolution gas chromatography, and magnetic sector mass spectrometry which gives detection limits in the low parts per trillion (ppt) range.

7. REGULATIONS AND ADVISORIES

National and State regulations and guidelines are listed in Table 7-1.

An acute-duration inhalation MRL for acetone of 26 ppm was derived. The MRL is based on a LOAEL value of 237 ppm for 4 hours for neurobehavioral effects in humans in an experimental study by Dick et al. (1989). Intermediate and chronic inhalation MRLs of 13 ppm were derived based on a LOAEL value of 1,250 ppm for neurological effects in humans in a 6-week study by Stewart et al. (1975).

An intermediate-duration oral MRL for acetone of 2 mg/kg/day was derived. The MRL is based on a NOAEL value of 200 mg/kg/day for macrocytic anemia in rats in the 13-week drinking water study (Dietz et al. 1991; NTP 1991). The LOAEL was 400 mg/kg/day.

EPA has verified a chronic oral reference dose (RfD) for acetone of 0.1 mg/kg/day (IRIS 1992). The RfD is based on the NOAEL of 100 mg/kg/day for increased liver and kidney weights and nephrotoxicity in rats in the 13-week gavage study by American Biogenics Corp. (1986).

TABLE 7-1. Regulations and Guidelines Applicable to Acetone

Agency	Description	Information	References
NATIONAL			
Regulations:			
a. Air:			
OSHA	PEL TWA	750 ppm (1,800 mg/m^3)	OSHA 1989 (29 CFR 1910.1000)
	STEL	1,000 ppm (2,400 mg/m^3)	
b. Other:			
EPA OERR	RQ	5,000 pounds	EPA 1985 (40 CFR 117 and 302)
Guidelines:			
a. Air:			
ACGIH	TLV TWA	750 ppm (1,780 mg/m^3)	ACGIH 1991
	STEL	1,000 ppm (2,380 mg/m^3)	
NIOSH	REL TWA	250 ppm (590 mg/m^3)	NIOSH 1992
b. Other:			
EPA	RfD (oral)	0.1 mg/kg/day	IRIS 1992
	Carcinogen classification	D[a]	IRIS 1992
STATE			
Regulations and Guidelines:			
a. Air:	Acceptable ambient air concentrations		NATICH 1992
Arizona		20.0 mg/m^3 (1-hour average)	
		14.0 mg/m^3 (24-hour average)	
Connecticut		11.8 mg/m^3 (8-hour average)	
Florida:			
(Tampa)		35.6 mg/m^3 (8-hour average)	
(Fort Lauderdale)		36.0 mg/m^3 (8-hour average)	
(Pinella)		35.6 mg/m^3 (8-hour average)	
(Pinella)		8.54 mg/m^3 (24-hour average)	
Massachusetts		0.161 mg/m^3 (24-hour average)	
		0.161 mg/m^3 (annual)	
Maine		3.5 mg/m^3 (24-hour average)	
		0.35 mg/m^3 (annual)	
North Dakota		17.8 mg/m^3 (8-hour average)	
		23.8 mg/m^3 (1-hour average)	
Nevada		42.4 mg/m^3 (8-hour average)	
New York		35.6 mg/m^3 (1-year average)	
South Dakota		11.8 mg/m^3 (8-hour average)	
Texas		5.9 mg/m^3 (30-minute average)	
		0.59 mg/m^3 (annual)	
Virginia		30.0 mg/m^3 (24-hour average)	
Vermont		178 mg/m^3 (8-hour average)	
b. Water:	Drinking water quality standards		FSTRAC 1990
Massachusetts		700 μg/L	
Maryland		3,600 μg/L	
Minnesota		700 μg/L	
New Hampshire		700 μg/L	

7. REGULATIONS AND ADVISORIES

TABLE 7-1. Regulations and Guidelines Applicable to Acetone *(continued)*

Agency	Description	Information	References
STATE *(continued)*			
standards for spent solvents			Treatment CELDS 1992 CaliforniaWaste
waters containing spent acetone	0.05 mg/L		All other
spent acetone wastes	0.59 mg/L		

[a]Not classifiable as to human carcinogenicity

ACGIH = American Conference of Governmental Industrial Hygientists; EPA = Environmental Protection Agency; NIOSH = National Institute for Occupational Safety and Health; OERR = Office of Emergency and Remedial Resonse; OSHA = Occupational Safety and Health Administration; PEL = Permissible Exposure Limit; REL = Recommended Exposure Limit; RfD = Reference Dose; RQ = Reportable Quantity; STEL = Short-term Exposure Limit; TLV = Threshold Limit Value; TWA = Time-weighted Average

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9. GLOSSARY

Acute Exposure - Exposure to a chemical for a duration of 14 days or less, as specified in the Toxicological Profiles.

Adsorption Coefficient (K_{oc}) - The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

Adsorption Ratio (Kd) - The amount of a chemical adsorbed by a sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

Bioconcentration Factor (BCF) - The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

Cancer Effect Level (CEL) - The lowest dose of chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or tumors) between the exposed population and its appropriate control.

Carcinogen - A chemical capable of inducing cancer.

Ceiling Value - A concentration of a substance that should not be exceeded, even instantaneously.

Chronic Exposure - Exposure to a chemical for 365 days or more, as specified in the Toxicological Profiles.

Developmental Toxicity -The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Embryotoxicity and Fetotoxicity - Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the insult occurred. The terms, as used here, include malformations and variations, altered growth, and in utero death.

EPA Health Advisory -An estimate of acceptable drinking water levels for a chemical substance based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

Immediately Dangerous to Life or Health (IDLH) - The maximum environmental concentration of a contaminant from which one could escape within 30 min without any escape-impairing symptoms or irreversible health effects.

Intermediate Exposure - Exposure to a chemical for a duration of 15-364 days, as specified in the Toxicological Profiles.

Immunologic Toxicity - The occurrence of adverse effects on the immune system that may result from exposure to environmental agents such as chemicals.

In Vitro - Isolated from the living organism and artificially maintained, as in a test tube.

In Vivo - Occurring within the living organism.

Lethal Concentration $_{(LO)}$ (LC_{LO}) - The lowest concentration of a chemical in air which has been reported to have caused death in humans or animals.

Lethal Concentration $_{(50)}$ (LC_{50}) - A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose$_{(LO)}$ (LD_{LO}) - The lowest dose of a chemical introduced by a route other than inhalation that is expected to have caused death in humans or animals.

Lethal Dose$_{(50)}$ (LD_{50}) - The dose of a chemical which has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time$_{(50)}$ (LT_{50}) - A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL) - The lowest dose of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Malformations - Permanent structural changes that may adversely affect survival, development, or function.

Minimal Risk Level - An estimate of daily human exposure to a dose of a chemical that is likely to be without an appreciable risk of adverse noncancerous effects over a specified duration of exposure.

Mutagen - A substance that causes mutations. A mutation is a change in the genetic material in a body cell. Mutations can lead to birth defects, miscarriages, or cancer.

Neurotoxicity - The occurrence of adverse effects on the nervous system following exposure to chemical.

No-Observed-Adverse-Effect Level (NOAEL) - The dose of chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Effects may be produced at this dose, but they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{OW}) - The equilibrium ratio of the concentrations of a chemical in n-octanol and water, in dilute solution.

Permissible Exposure Limit (PEL) - An allowable exposure level in workplace air averaged over an 8-hour shift.

q_1*- The upper-bound estimate of the low-dose slope of the dose-response curve as determined by the multistage procedure. The ql* can be used to calculate an estimate of carcinogenic potency, the incremental excess cancer risk per unit of exposure (usually ug/L for water, mg/kg/day for food, and ug/m^3 for air).

Reference Dose (RfD) - An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure of the human population to a potential hazard that is likely to be without risk of deleterious effects during a lifetime. The RfD is operationally derived from the NOAEL (from animal and human studies) by a consistent application of uncertainty factors that reflect various types of data used to estimate RfDs and an additional modifying factor, which is based on a professional judgment of the entire database on the chemical. The RfDs are not applicable to nonthreshold effects such as cancer.

Reportable Quantity (RQ) - The quantity of a hazardous substance that is considered reportable under CERCLA. Reportable quantities are (1) 1 pound or greater or (2) for selected substances, an amount established by regulation either under CERCLA or under Sect. 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

Reproductive Toxicity - The occurrence of adverse effects on the reproductive system that may result from exposure to a chemical. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

RC_{50} - The calculated concentration that decreases the respiratory rate by 50%.

Short-Term Exposure Limit (STEL) - The maximum concentration to which workers can be exposed for up to 15 min continually. No more than four excursions are allowed per day, and there must be at least 60 min between exposure periods. The daily TLV-TWA may not be exceeded.

Target Organ Toxicity - This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen - A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV) - A concentration of a substance to which most workers can be exposed without adverse effect. The TLV may be expressed as a TWA, as a STEL, or as a CL.

Time-Weighted Average (TWA) - An allowable exposure concentration averaged over a normal 8-hour workday or 40-hour workweek.

Toxic Dose (TD_{50}) - A calculated dose of a chemical, introduced by a route other than inhalation, which is expected to cause a specific toxic effect in 50% of a defined experimental animal population.

Uncertainty Factor (UF) - A factor used in operationally deriving the RfD from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using LOAEL data rather than NOAEL data. Usually each of these factors is set equal to 10.

APPENDIX A

USER'S GUIDE

Chapter 1

Public Health Statement

This chapter of the profile is a health effects summary written in nontechnical language. Its intended audience is the general public especially people living in the vicinity of a hazardous waste site or substance release. If the Public Health Statement were removed from the rest of the document, it would still communicate to the lay public essential information about the substance.

The major headings in the Public Health Statement are useful to find specific topics of concern. The topics are written in a question and answer format. The answer to each question includes a sentence that will direct the reader to chapters in the profile that will provide more information on the given topic.

Chapter 2

Tables and Figures for Levels of Significant Exposure (LSE)

Tables (2-1, 2-2, and 2-3) and figures (2-l and 2-2) are used to summarize health effects by duration of exposure and end point and to illustrate graphically levels of exposure associated with those effects. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of No-Observed-Adverse-Effect Levels (NOAELs), Lowest-Observed-Adverse-Effect Levels (LOAELs) for Less Serious and Serious health effects, or Cancer Effect Levels (CELs). In addition, these tables and figures illustrate differences in response by species, Minimal Risk Levels (MRLs) to humans for noncancer end points, and EPA's estimated range associated with an upper-bound individual lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. The LSE tables and figures can be used for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text.

The legends presented below demonstrate the application of these tables and figures. A representative example of LSE Table 2-1 and Figure 2-l are shown. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

LEGEND

See LSE Table 2-1

(1). Route of Exposure One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. When sufficient data exist, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure, i.e., inhalation, oral, and dermal (LSE Table 2-1, 2-2, and 2-3, respectively). LSE figures are limited to the inhalation (LSE Figure 2-1) and oral (LSE Figure 2-2) routes.

(2). Exposure Duration Three exposure periods: acute (14 days or less); intermediate (15 to 364 days); and chronic (365 days or more) are presented within each route of exposure. In this example, an inhalation study of intermediate duration exposure is reported.

(3). Health Effect The major categories of health effects included in LSE tables and figures are death, systemic, immunological, neurological, developmental, reproductive, and cancer. NOAELs and LOAELs can be reported in the tables and figures for all effects but cancer. Systemic effects are further defined in the "System" column of the LSE table.

(4). Key to Figure Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 18 has been used to define a NOAEL and a Less Serious LOAEL (also see the two "18r" data points in Figure 2-l).

(5). Species The test species, whether animal or human, are identified in this column.

(6). Exposure Frequency/Duration The duration of the study and the weekly and daily exposure regimen are provided in this column. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 18), rats were exposed to [substance x] via inhalation for 13 weeks, 5 days per week, for 6 hours per day.

(7). System This column further defmes the systemic effects. These systems include: respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, and dermal/ocular. "Other" refers to any systemic effect (e.g., a decrease in body weight) not covered in these systems. In the example of key number 18, one systemic effect (respiratory) was investigated in this study.

(8) NOAEL A No-Observed-Adverse-Effect Level (NOAEL) is the highest exposure level at which no harmful effects were seen in the organ system studied. Key number 18 reports a NOAEL of 3 ppm for the respiratory system which was used to derive an intermediate exposure, inhalation MRL of 0.005 ppm (see footnote "b").

(9). LOAEL A Lowest-Observed-Adverse-Effect Level (LOAEL) is the lowest exposure level used in the study that caused a harmful health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific end point used to quantify the adverse effect accompanies the LOAEL. The "Less Serious" respiratory effect reported in key number 18 (hyperplasia) occurred at a LOAEL of 10 ppm.

(10). Reference The complete reference citation is given in Chapter 8 of the profile.

(11). CEL A Cancer Effect Level (CEL) is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiological studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses which did not cause a measurable increase in cancer.

(12.) Footnotes Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. Footnote "b" indicates the NOAEL of 3 ppm in key number 18 was used to derive an MRL of 0.005 ppm.

TABLE 2-1. Levels of Significant Exposure to [Chemical x] - Inhalation

Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
INTERMEDIATE EXPOSURE							
Systemic							
18	Rat	13 wk 5d/wk 6hr/d	Resp	3[b]	10 (hyperplasia)		Nitschke et al. 1981
CHRONIC EXPOSURE							
Cancer							
38	Rat	18 mo 5d/wk 7hr/d				20 (CEL, multiple organs)	Wong et al. 1982
39	Rat	89-104 wk 5d/wk 6hr/d				10 (CEL, lung tumors, nasal tumors)	NTP 1982
40	Mouse	79-103 wk 5d/wk 6hr/d				10 (CEL, lung tumors, hemangiosarcomas)	NTP 1982

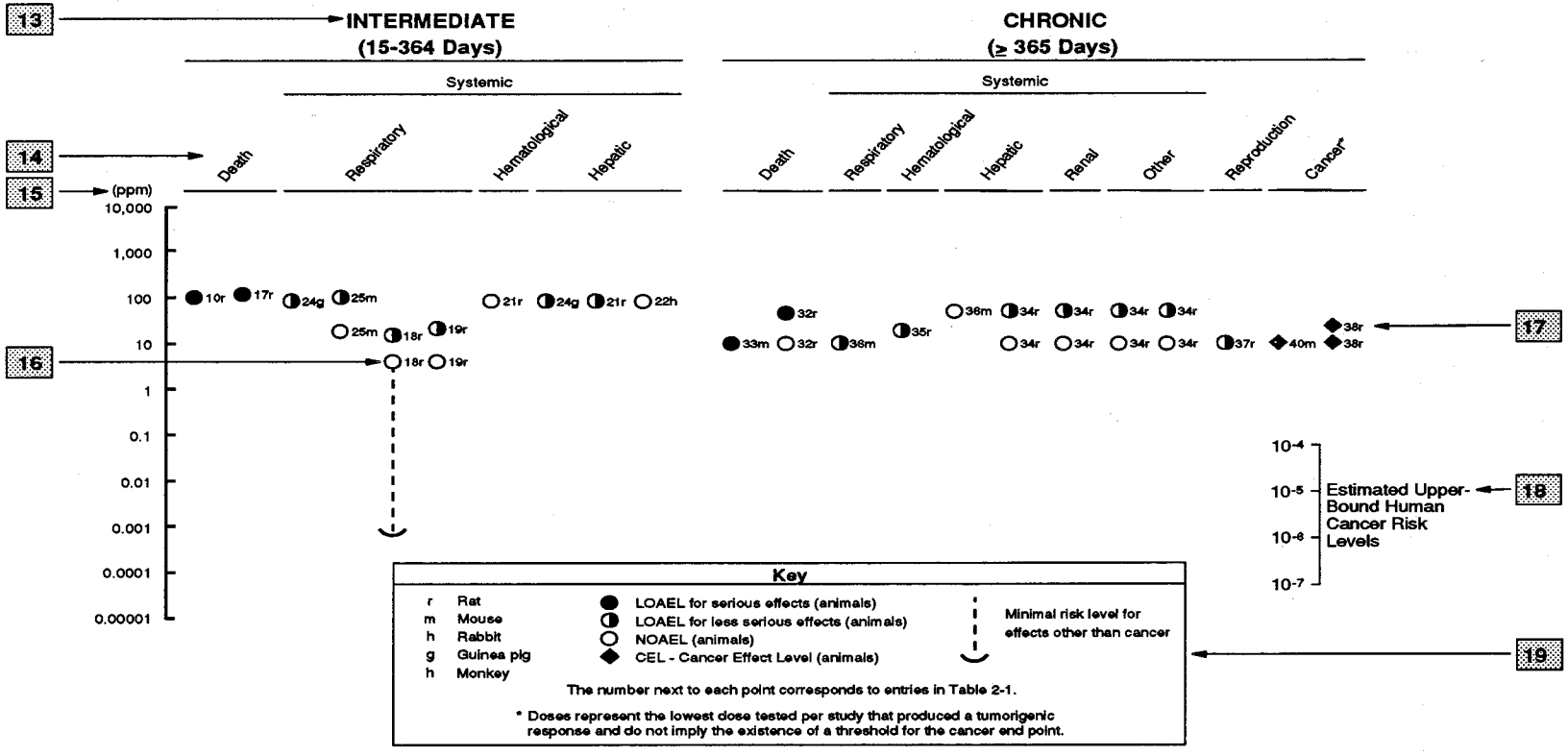
[a] The number corresponds to entries in Figure 2-1.

[b] Used to derive an intermediate inhalation Minimal Risk Level (MRL) of 5 x 10^{-3} ppm; dose adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animal to humans, 10 for human variability).

CEL = cancer effect level; d = day(s); hr = hour(s); LOAEL = lowest-observed-adverse-effect level; mo = month(s); NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s)

SAMPLE

FIGURE 2-1. Levels of Significant Exposure to [Chemical X] - Inhalation



(13). Exposure Duration The same exposure periods appear as in the LSE table. In this example, health effects observed within the intermediate and chronic exposure periods are illustrated.

(14). Health Effect These are the categories of health effects for which reliable quantitative data exist. The same health effects appear in the LSE table.

(15). Levels of Exposure Exposure levels for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure levels are reported on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(16). NOAEL In this example, 18r NOAEL is the critical end point for which an intermediate inhalation exposure MRL is based. As you can see from the LSE figure key, the open-circle symbol indicates a NOAEL for the test species (rat). The key number 18 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 3 ppm (see entry 18 in the Table) to the MRL of 0.005 ppm (see footnote "b" in the LSE table).

(17). CEL Key number 38r is one of three studies for which Cancer Effect Levels (CELs) were derived. The diamond symbol refers to a CEL for the test species (rat). The number 38 corresponds to the entry in the LSE table.

(18). Estimated Upper-Bound Human Cancer Risk Levels This is the range associated with the upper-bound for lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. These risk levels are derived from EPA's Human Health Assessment Group's upper-bound estimates of the slope of the cancer dose response curve at low dose levels (q_1*).

(19). Key to LSE Figure The Key explains the abbreviations and symbols used in the figure.

Chapter 2 (Section 2.4)

Relevance to Public Health

The Relevance to Public Health section provides a health effects summary based on evaluations of existing toxicological, epidemiological, and toxicokinetic information. This summary is designed to present interpretive, weight-of-evidence discussions for human health end points by addressing the following questions.

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

The section discusses health effects by end point. Human data are presented first, then animal data. Both are organized by route of exposure (inhalation, oral, and dermal) and by duration (acute, intermediate, and chronic). *In vitro* data and data from parenteral routes (intramuscular, intravenous, subcutaneous, etc.) are also considered in this section. If data are located in the scientific literature, a table of genotoxicity information is included.

The carcinogenic potential of the profiled substance is qualitatively evaluated, when appropriate, using existing toxicokinetic, genotoxic, and carcinogenic data. ATSDR does not currently assess cancer potency or perform cancer risk assessments. Marls for noncancer end points if derived, and the end points from which they were derived are indicated and discussed in the appropriate section(s).

Limitations to existing scientific literature that prevent a satisfactory evaluation of the relevance to public health are identified in the Identification of Data Needs section.

Interpretation of Minimal Risk Levels

Where sufficient toxicologic information was available, MRLs were derived. MRLs are specific for route (inhalation or oral) and duration (acute, intermediate, or chronic) of exposure. Ideally, MRLs can be derived from all six exposure scenarios (e.g., Inhalation - acute, -intermediate, -chronic; Oral - acute, -intermediate, - chronic). These MRLs are not meant to support regulatory action, but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans. They should help physicians and public health officials determine the safety of a community living near a substance emission, given the concentration of a contaminant in air or the estimated daily dose received via food or water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicological information on which the number is based. Section 2.4, “Relevance to Public Health,” contains basic information known about the substance. Other sections such as 2.6, “Interactions with Other Chemicals” and 2.7, “Populations that are Unusually Susceptible” provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology used by the Environmental Protection Agency (EPA) (Barnes and Dourson 1988; EPA 1989a) to derive reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the end point which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential effects (e.g., systemic, neurological, and developmental). In order to compare NOAELs and LOAELs for specific end points, all inhalation exposure levels are adjusted for 24hr exposures and all intermittent exposures for inhalation and oral routes of intermediate and chronic duration are adjusted for continuous exposure (i.e., 7 days/week). If the information and reliable quantitative data on the chosen end point are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest NOAEL that does not exceed any adverse effect levels. The NOAEL is the most suitable end point for deriving an MRL. When a NOAEL is not available, a Less Serious LOAEL can be used to derive an MRL, and an uncertainty factor of (1, 3, or 10) is employed. MRLs are not derived from Serious LOAELs. Additional uncertainty factors of (1, 3, or 10) are used for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and (1, 3, or 10) are used for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. Generally an uncertainty factor of 10 is used; however, the MRL Workgroup reserves the right to use uncertainty factors of (1, 3, or 10) based on scientific judgement. The product is then divided into the adjusted inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a substance-specific MRL are provided in the footnotes of the LSE Tables.

APPENDIX B

ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
ADME	Absorption, Distribution, Metabolism, and Excretion
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
BCF	bioconcentration factor
BSC	Board of Scientific Counselors
C	Centigrade
CDC	Centers for Disease Control
CEL	Cancer Effect Level
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
CLP	Contract Laboratory Program
cm	centimeter
CNS	central nervous system
d	day
DHEW	Department of Health, Education, and Welfare
DHHS	Department of Health and Human Services
DOL	Department of Labor
ECG	electrocardiogram
EEG	electroencephalogram
EPA	Environmental Protection Agency
EKG	see ECG
F	Fahrenheit
F_1	first filial generation
FAO	Food and Agricultural Organization of the United Nations
FEMA	Federal Emergency Management Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
fpm	feet per minute
ft	foot
FR	Federal Register
g	gram
GC	gas chromatography
gen	generation
HPLC	high-n-performance liquid chromatography
hr	hour
IDLH	Immediately Dangerous to Life and Health
IARC	International Agency for Research on Cancer
ILO	International Labor Organization
in	inch
Kd	adsorption ratio
kg	kilogram
kkg	metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient

L	liter
LC	liquid chromatography
LC_{Lo}	lethal concentration, low
LC_{50}	lethal concentration, 50% kill
LD_{Lo}	lethal dose, low
LD_{50}	lethal dose, 50% kill
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
m	meter
mg	milligram
min	minute
mL	milliliter
mm	millimeter
mmHg	millimeters of mercury
mmol	millimole
mo	month
mppcf	millions of particles per cubic foot
MRL	Minimal Risk Level
MS	mass spectrometry
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NIOSHTIC	NIOSH's Computerized Information Retrieval System
ng	nanogram
nm	nanometer
NHANES	National Health and Nutrition Examination Survey
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NOES	National Occupational Exposure Survey
NOHS	National Occupational Hazard Survey
NPL	National Priorities List
NRC	National Research Council
NTIS	National Technical Information Service
NTP	National Toxicology Program
OSHA	Occupational Safety and Health Administration
PEL	permissible exposure limit
pg	picogram
pmol	picomole
PHS	Public Health Service
PMR	proportionate mortality ratio
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
REL	recommended exposure limit
RfD	Reference Dose
RTECS	Registry of Toxic Effects of Chemical Substances
sec	second
SCE	sister chromatid exchange
SIC	Standard Industrial Classification
SMR	standard mortality ratio

APPENDIX B

STEL	short term exposure limit
STORET	STORAGE and RETRIEVAL
TLV	threshold limit value
TSCA	Toxic Substances Control Act
TRI	Toxics Release Inventory
TWA	time-weighted average
U.S.	United States
UF	uncertainty factor
yr	year
WHO	World Health Organization
wk	week
>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to
%	percent
α	alpha
β	beta
δ	delta
γ	gamma
μm	micron
μg	microgram

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TOXICOLOGICAL PROFILE FOR ARSENIC

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

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DISCLAIMER

The use of company or product name(s) is for identification only and does not imply endorsement by the Agency for Toxic Substances and Disease Registry.

UPDATE STATEMENT

A Toxicological Profile for Arsenic, Draft for Public Comment was released in September 2005. This edition supersedes any previously released draft or final profile.

Toxicological profiles are revised and republished as necessary. For information regarding the update status of previously released profiles, contact ATSDR at:

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FOREWORD

This toxicological profile is prepared in accordance with guidelines developed by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA). The original guidelines were published in the *Federal Register* on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for the hazardous substance described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a hazardous substance's toxicologic properties. Other pertinent literature is also presented, but is described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

The focus of the profiles is on health and toxicologic information; therefore, each toxicological profile begins with a public health statement that describes, in nontechnical language, a substance's relevant toxicological properties. Following the public health statement is information concerning levels of significant human exposure and, where known, significant health effects. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to protection of public health are identified by ATSDR and EPA.

Each profile includes the following:

(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a hazardous substance to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects;

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure that present a significant risk to human health of acute, subacute, and chronic health effects; and

(C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

The principal audiences for the toxicological profiles are health professionals at the Federal, State, and local levels; interested private sector organizations and groups; and members of the public.

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed. Staff of the Centers for Disease Control and Prevention and other Federal scientists have also reviewed the profile. In addition, this profile has been peer-reviewed by a nongovernmental panel and is being made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

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The toxicological profiles are developed in response to the Superfund Amendments and Reauthorization Act (SARA) of 1986 (Public Law 99-499) which amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). This public law directed ATSDR to prepare toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. The availability of the revised priority list of 275 hazardous substances was announced in the *Federal Register* on December 7, 2005 (70 FR 72840). For prior versions of the list of substances, see *Federal Register* notices dated April 17, 1987 (52 FR 12866); October 20, 1988 (53 FR 41280); October 26, 1989 (54 FR 43619); October 17, 1990 (55 FR 42067); October 17, 1991 (56 FR 52166); October 28, 1992 (57 FR 48801); February 28, 1994 (59 FR 9486); April 29, 1996 (61 FR 18744); November 17, 1997 (62 FR 61332); October 21, 1999 (64 FR 56792); October 25, 2001 (66 FR 54014); and November 7, 2003 (68 FR 63098). Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list.

QUICK REFERENCE FOR HEALTH CARE PROVIDERS

Toxicological Profiles are a unique compilation of toxicological information on a given hazardous substance. Each profile reflects a comprehensive and extensive evaluation, summary, and interpretation of available toxicologic and epidemiologic information on a substance. Health care providers treating patients potentially exposed to hazardous substances will find the following information helpful for fast answers to often-asked questions.

Primary Chapters/Sections of Interest

Chapter 1: Public Health Statement: The Public Health Statement can be a useful tool for educating patients about possible exposure to a hazardous substance. It explains a substance's relevant toxicologic properties in a nontechnical, question-and-answer format, and it includes a review of the general health effects observed following exposure.

Chapter 2: Relevance to Public Health: The Relevance to Public Health Section evaluates, interprets, and assesses the significance of toxicity data to human health.

Chapter 3: Health Effects: Specific health effects of a given hazardous compound are reported by type of health effect (death, systemic, immunologic, reproductive), by route of exposure, and by length of exposure (acute, intermediate, and chronic). In addition, both human and animal studies are reported in this section.
NOTE: Not all health effects reported in this section are necessarily observed in the clinical setting. Please refer to the Public Health Statement to identify general health effects observed following exposure.

Pediatrics: Four new sections have been added to each Toxicological Profile to address child health issues:

Section 1.6 How Can (Chemical X) Affect Children?
Section 1.7 How Can Families Reduce the Risk of Exposure to (Chemical X)?
Section 3.7 Children's Susceptibility
Section 6.6 Exposures of Children

Other Sections of Interest:

Section 3.8 Biomarkers of Exposure and Effect
Section 3.11 Methods for Reducing Toxic Effects

ATSDR Information Center

Phone: 1-800-CDC-INFO (800-232-4636) or 1-888-232-6348 (TTY) ***Fax:*** (770) 488-4178
E-mail: cdcinfo@cdc.gov ***Internet***: http://www.atsdr.cdc.gov

The following additional material can be ordered through the ATSDR Information Center:

Case Studies in Environmental Medicine: Taking an Exposure History—The importance of taking an exposure history and how to conduct one are described, and an example of a thorough exposure history is provided. Other case studies of interest include *Reproductive and Developmental*

Hazards; Skin Lesions and Environmental Exposures; Cholinesterase-Inhibiting Pesticide Toxicity; and numerous chemical-specific case studies.

Managing Hazardous Materials Incidents is a three-volume set of recommendations for on-scene (prehospital) and hospital medical management of patients exposed during a hazardous materials incident. Volumes I and II are planning guides to assist first responders and hospital emergency department personnel in planning for incidents that involve hazardous materials. Volume III—*Medical Management Guidelines for Acute Chemical Exposures*—is a guide for health care professionals treating patients exposed to hazardous materials.

Fact Sheets (ToxFAQs) provide answers to frequently asked questions about toxic substances.

Other Agencies and Organizations

The National Center for Environmental Health (NCEH) focuses on preventing or controlling disease, injury, and disability related to the interactions between people and their environment outside the workplace. Contact: NCEH, Mailstop F-29, 4770 Buford Highway, NE, Atlanta, GA 30341-3724 • Phone: 770-488-7000 • FAX: 770-488-7015.

The National Institute for Occupational Safety and Health (NIOSH) conducts research on occupational diseases and injuries, responds to requests for assistance by investigating problems of health and safety in the workplace, recommends standards to the Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA), and trains professionals in occupational safety and health. Contact: NIOSH, 200 Independence Avenue, SW, Washington, DC 20201 • Phone: 800-356-4674 or NIOSH Technical Information Branch, Robert A. Taft Laboratory, Mailstop C-19, 4676 Columbia Parkway, Cincinnati, OH 45226-1998 • Phone: 800-35-NIOSH.

The National Institute of Environmental Health Sciences (NIEHS) is the principal federal agency for biomedical research on the effects of chemical, physical, and biologic environmental agents on human health and well-being. Contact: NIEHS, PO Box 12233, 104 T.W. Alexander Drive, Research Triangle Park, NC 27709 • Phone: 919-541-3212.

Referrals

The Association of Occupational and Environmental Clinics (AOEC) has developed a network of clinics in the United States to provide expertise in occupational and environmental issues. Contact: AOEC, 1010 Vermont Avenue, NW, #513, Washington, DC 20005 • Phone: 202-347-4976 • FAX: 202-347-4950 • e-mail: AOEC@AOEC.ORG • Web Page: http://www.aoec.org/.

The American College of Occupational and Environmental Medicine (ACOEM) is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. Contact: ACOEM, 25 Northwest Point Boulevard, Suite 700, Elk Grove Village, IL 60007-1030 • Phone: 847-818-1800 • FAX: 847-818-9266.

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THE PROFILE HAS UNDERGONE THE FOLLOWING ATSDR INTERNAL REVIEWS:

1. Health Effects Review. The Health Effects Review Committee examines the health effects chapter of each profile for consistency and accuracy in interpreting health effects and classifying end points.

2. Minimal Risk Level Review. The Minimal Risk Level Workgroup considers issues relevant to substance-specific Minimal Risk Levels (MRLs), reviews the health effects database of each profile, and makes recommendations for derivation of MRLs.

3. Data Needs Review. The Applied Toxicology Branch reviews data needs sections to assure consistency across profiles and adherence to instructions in the Guidance.

4. Green Border Review. Green Border review assures the consistency with ATSDR policy.

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PEER REVIEW

A peer review panel was assembled for arsenic. The panel consisted of the following members:

1. Toby Rossman, Ph.D., Professor of Environmental Medicine, New York University School of Medicine, Nelson Institute of Environmental Medicine, Tuxedo, New York;

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These experts collectively have knowledge of arsenic's physical and chemical properties, toxicokinetics, key health end points, mechanisms of action, human and animal exposure, and quantification of risk to humans. All reviewers were selected in conformity with the conditions for peer review specified in Section 104(I)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

Scientists from the Agency for Toxic Substances and Disease Registry (ATSDR) have reviewed the peer reviewers' comments and determined which comments will be included in the profile. A listing of the peer reviewers' comments not incorporated in the profile, with a brief explanation of the rationale for their exclusion, exists as part of the administrative record for this compound.

The citation of the peer review panel should not be understood to imply its approval of the profile's final content. The responsibility for the content of this profile lies with the ATSDR.

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1. PUBLIC HEALTH STATEMENT

This public health statement tells you about arsenic and the effects of exposure to it.

The Environmental Protection Agency (EPA) identifies the most serious hazardous waste sites in the nation. These sites are then placed on the National Priorities List (NPL) and are targeted for long-term federal clean-up activities. Arsenic has been found in at least 1,149 of the 1,684 current or former NPL sites. Although the total number of NPL sites evaluated for this substance is not known, the possibility exists that the number of sites at which arsenic is found may increase in the future as more sites are evaluated. This information is important because these sites may be sources of exposure and exposure to this substance may harm you.

When a substance is released either from a large area, such as an industrial plant, or from a container, such as a drum or bottle, it enters the environment. Such a release does not always lead to exposure. You can be exposed to a substance only when you come in contact with it. You may be exposed by breathing, eating, or drinking the substance, or by skin contact.

If you are exposed to arsenic, many factors will determine whether you will be harmed. These factors include the dose (how much), the duration (how long), and how you come in contact with it. You must also consider any other chemicals you are exposed to and your age, sex, diet, family traits, lifestyle, and state of health.

1.1 WHAT IS ARSENIC?

Arsenic is a naturally occurring element that is widely distributed in the Earth's crust. Arsenic is classified chemically as a metalloid, having both properties of a metal and a nonmetal; however, it is frequently referred to as a metal. Elemental arsenic (sometimes referred to as metallic arsenic) is a steel grey solid material. However, arsenic is usually found in the environment combined with other elements such as oxygen, chlorine, and sulfur. Arsenic combined with

these elements is called inorganic arsenic. Arsenic combined with carbon and hydrogen is referred to as organic arsenic.

Most inorganic and organic arsenic compounds are white or colorless powders that do not evaporate. They have no smell, and most have no special taste. Thus, you usually cannot tell if arsenic is present in your food, water, or air.

Inorganic arsenic occurs naturally in soil and in many kinds of rock, especially in minerals and ores that contain copper or lead. When these ores are heated in smelters, most of the arsenic goes up the stack and enters the air as a fine dust. Smelters may collect this dust and take out the arsenic as a compound called arsenic trioxide (As_2O_3). However, arsenic is no longer produced in the United States; all of the arsenic used in the United States is imported.

Presently, about 90% of all arsenic produced is used as a preservative for wood to make it resistant to rotting and decay. The preservative is copper chromated arsenate (CCA) and the treated wood is referred to as “pressure-treated.” In 2003, U.S. manufacturers of wood preservatives containing arsenic began a voluntary transition from CCA to other wood preservatives that do not contain arsenic in wood products for certain residential uses, such as play structures, picnic tables, decks, fencing, and boardwalks. This phase out was completed on December 31, 2003; however, wood treated prior to this date could still be used and existing structures made with CCA-treated wood would not be affected. CCA-treated wood products continue to be used in industrial applications. It is not known whether, or to what extent, CCA-treated wood products may contribute to exposure of people to arsenic.

In the past, inorganic arsenic compounds were predominantly used as pesticides, primarily on cotton fields and in orchards. Inorganic arsenic compounds can no longer be used in agriculture. However, organic arsenic compounds, namely cacodylic acid, disodium methylarsenate (DSMA), and monosodium methylarsenate (MSMA), are still used as pesticides, principally on cotton. Some organic arsenic compounds are used as additives in animal feed. Small quantities of elemental arsenic are added to other metals to form metal mixtures or alloys with improved

properties. The greatest use of arsenic in alloys is in lead-acid batteries for automobiles. Another important use of arsenic compounds is in semiconductors and light-emitting diodes.

To learn more about the properties and uses of arsenic, see Chapters 4 and 5.

1.2 WHAT HAPPENS TO ARSENIC WHEN IT ENTERS THE ENVIRONMENT?

Arsenic occurs naturally in soil and minerals and it therefore may enter the air, water, and land from wind-blown dust and may get into water from runoff and leaching. Volcanic eruptions are another source of arsenic. Arsenic is associated with ores containing metals, such as copper and lead. Arsenic may enter the environment during the mining and smelting of these ores. Small amounts of arsenic also may be released into the atmosphere from coal-fired power plants and incinerators because coal and waste products often contain some arsenic.

Arsenic cannot be destroyed in the environment. It can only change its form, or become attached to or separated from particles. It may change its form by reacting with oxygen or other molecules present in air, water, or soil, or by the action of bacteria that live in soil or sediment. Arsenic released from power plants and other combustion processes is usually attached to very small particles. Arsenic contained in wind-borne soil is generally found in larger particles. These particles settle to the ground or are washed out of the air by rain. Arsenic that is attached to very small particles may stay in the air for many days and travel long distances. Many common arsenic compounds can dissolve in water. Thus, arsenic can get into lakes, rivers, or underground water by dissolving in rain or snow or through the discharge of industrial wastes. Some of the arsenic will stick to particles in the water or sediment on the bottom of lakes or rivers, and some will be carried along by the water. Ultimately, most arsenic ends up in the soil or sediment. Although some fish and shellfish take in arsenic, which may build up in tissues, most of this arsenic is in an organic form called arsenobetaine (commonly called "fish arsenic") that is much less harmful.

For more information on how arsenic behaves in the environment, see Chapter 6.

1.3 HOW MIGHT I BE EXPOSED TO ARSENIC?

Since arsenic is found naturally in the environment, you will be exposed to some arsenic by eating food, drinking water, or breathing air. Children may also be exposed to arsenic by eating soil. Analytical methods used by scientists to determine the levels of arsenic in the environment generally do not determine the specific form of arsenic present. Therefore, we do not always know the form of arsenic a person may be exposed to. Similarly, we often do not know what forms of arsenic are present at hazardous waste sites. Some forms of arsenic may be so tightly attached to particles or embedded in minerals that they are not taken up by plants and animals.

The concentration of arsenic in soil varies widely, generally ranging from about 1 to 40 parts of arsenic to a million parts of soil (ppm) with an average level of 3–4 ppm. However, soils in the vicinity of arsenic-rich geological deposits, some mining and smelting sites, or agricultural areas where arsenic pesticides had been applied in the past may contain much higher levels of arsenic. The concentration of arsenic in natural surface and groundwater is generally about 1 part in a billion parts of water (1 ppb), but may exceed 1,000 ppb in contaminated areas or where arsenic levels in soil are high. Groundwater is far more likely to contain high levels of arsenic than surface water. Surveys of U.S. drinking water indicate that about 80% of water supplies have less than 2 ppb of arsenic, but 2% of supplies exceed 20 ppb of arsenic. Levels of arsenic in food range from about 20 to 140 ppb. However, levels of inorganic arsenic, the form of most concern, are far lower. Levels of arsenic in the air generally range from less than 1 to about 2,000 nanograms (1 nanogram equals a billionth of a gram) of arsenic per cubic meter of air (less than 1–2,000 ng/m^3), depending on location, weather conditions, and the level of industrial activity in the area. However, urban areas generally have mean arsenic levels in air ranging from 20 to 30 ng/m^3.

You normally take in small amounts of arsenic in the air you breathe, the water you drink, and the food you eat. Of these, food is usually the largest source of arsenic. The predominant dietary source of arsenic is seafood, followed by rice/rice cereal, mushrooms, and poultry. While seafood contains the greatest amounts of arsenic, for fish and shellfish, this is mostly in an organic form of arsenic called arsenobetaine that is much less harmful. Some seaweeds may

contain arsenic in inorganic forms that may be more harmful. Children are likely to eat small amounts of dust or soil each day, so this is another way they may be exposed to arsenic. The total amount of arsenic you take in from these sources is generally about 50 micrograms (1 microgram equals one-millionth of a gram) each day. The level of inorganic arsenic (the form of most concern) you take in from these sources is generally about 3.5 microgram/day. Children may be exposed to small amounts of arsenic from hand-to-mouth activities from playing on play structures or decks constructed out of CCA-treated wood. The potential exposure that children may receive from playing in play structures constructed from CCA-treated wood is generally smaller than that they would receive from food and water. Hand washing can reduce the potential exposure of children to arsenic after playing on play structures constructed with CCA-treated wood, since most of the arsenic on the children's hands was removed with water.

In addition to the normal levels of arsenic in air, water, soil, and food, you could be exposed to higher levels in several ways, such as the following:

- Some areas of the United States contain unusually high natural levels of arsenic in rock, and this can lead to unusually high levels of arsenic in soil or water. If you live in an area like this, you could take in elevated amounts of arsenic in drinking water. Children may be taking in higher amounts of arsenic because of hand-to-mouth contact or eating soil in areas with higher than usual arsenic concentrations.

- Some hazardous waste sites contain large quantities of arsenic. If the material is not properly disposed of, it can get into surrounding water, air, or soil. If you live near such a site, you could be exposed to elevated levels of arsenic from these media.

- If you work in an occupation that involves arsenic production or use (for example, copper or lead smelting, wood treating, or pesticide application), you could be exposed to elevated levels of arsenic during your work.

- If you saw or sand arsenic-treated wood, you could inhale some of the sawdust into your nose or throat. Similarly, if you burn arsenic-treated wood, you could inhale arsenic in the smoke.

- If you live in a former agricultural area where arsenic was used on crops, the soil could contain high levels of arsenic.

- In the past, several kinds of products used in the home (rat poison, ant poison, weed killer, some types of medicines) had arsenic in them. However, most of these uses of arsenic have ended, so you are not likely to be exposed from home products any longer.

You can find more information on how you may be exposed to arsenic in Chapter 6.

1.4 HOW CAN ARSENIC ENTER AND LEAVE MY BODY?

If you swallow arsenic in water, soil, or food, most of the arsenic may quickly enter into your body. The amount that enters your body will depend on how much you swallow and the kind of arsenic that you swallow. This is the most likely way for you to be exposed near a waste site. If you breathe air that contains arsenic dusts, many of the dust particles settle onto the lining of the lungs. Most of the arsenic in these particles is then taken up from the lungs into the body. You might be exposed in this way near waste sites where arsenic-contaminated soils are allowed to blow into the air, or if you work with arsenic-containing soil or products. If you get arsenic-contaminated soil or water on your skin, only a small amount will go through your skin into your body, so this is usually not of concern.

Both inorganic and organic forms leave your body in your urine. Most of the inorganic arsenic will be gone within several days, although some will remain in your body for several months or even longer. If you are exposed to organic arsenic, most of it will leave your body within several days.

You can find more information on how arsenic enters and leaves your body in Chapter 3.

1.5 HOW CAN ARSENIC AFFECT MY HEALTH?

Scientists use many tests to protect the public from harmful effects of toxic chemicals and to find ways for treating persons who have been harmed.

One way to learn whether a chemical will harm people is to determine how the body absorbs, uses, and releases the chemical. For some chemicals, animal testing may be necessary. Animal testing may also help identify health effects such as cancer or birth defects. Without laboratory animals, scientists would lose a basic method for getting information needed to make wise decisions that protect public health. Scientists have the responsibility to treat research animals with care and compassion. Scientists must comply with strict animal care guidelines because laws today protect the welfare of research animals.

Inorganic arsenic has been recognized as a human poison since ancient times, and large oral doses (above 60,000 ppb in water which is 10,000 times higher than 80% of U.S. drinking water arsenic levels) can result in death. If you swallow lower levels of inorganic arsenic (ranging from about 300 to 30,000 ppb in water; 100–10,000 times higher than most U.S. drinking water levels), you may experience irritation of your stomach and intestines, with symptoms such as stomachache, nausea, vomiting, and diarrhea. Other effects you might experience from swallowing inorganic arsenic include decreased production of red and white blood cells, which may cause fatigue, abnormal heart rhythm, blood-vessel damage resulting in bruising, and impaired nerve function causing a "pins and needles" sensation in your hands and feet.

Perhaps the single-most characteristic effect of long-term oral exposure to inorganic arsenic is a pattern of skin changes. These include patches of darkened skin and the appearance of small "corns" or "warts" on the palms, soles, and torso, and are often associated with changes in the blood vessels of the skin. Skin cancer may also develop. Swallowing arsenic has also been reported to increase the risk of cancer in the liver, bladder, and lungs. The Department of Health and Human Services (DHHS) has determined that inorganic arsenic is known to be a human carcinogen (a chemical that causes cancer). The International Agency for Research on Cancer (IARC) has determined that inorganic arsenic is carcinogenic to humans. EPA also has classified inorganic arsenic as a known human carcinogen.

If you breathe high levels of inorganic arsenic, then you are likely to experience a sore throat and irritated lungs. You may also develop some of the skin effects mentioned above. The exposure level that produces these effects is uncertain, but it is probably above 100 micrograms of arsenic

per cubic meter ($\mu g/m^3$) for a brief exposure. Longer exposure at lower concentrations can lead to skin effects, and also to circulatory and peripheral nervous disorders. There are some data suggesting that inhalation of inorganic arsenic may also interfere with normal fetal development, although this is not certain. An important concern is the ability of inhaled inorganic arsenic to increase the risk of lung cancer. This has been seen mostly in workers exposed to arsenic at smelters, mines, and chemical factories, but also in residents living near smelters and arsenical chemical factories. People who live near waste sites with arsenic may have an increased risk of lung cancer as well.

If you have direct skin contact with high concentrations of inorganic arsenic compounds, your skin may become irritated, with some redness and swelling. However, it does not appear that skin contact is likely to lead to any serious internal effects.

Almost no information is available on the effects of organic arsenic compounds in humans. Studies in animals show that most simple organic arsenic compounds (such as methyl and dimethyl compounds) are less toxic than the inorganic forms. In animals, ingestion of methyl compounds can result in diarrhea, and lifetime exposure can damage the kidneys. Lifetime exposure to dimethyl compounds can damage the urinary bladder and the kidneys.

You can find more information on the health effects of inorganic and organic arsenic in Chapters 2 and 3.

1.6 HOW CAN ARSENIC AFFECT CHILDREN?

This section discusses potential health effects in humans from exposures during the period from conception to maturity at 18 years of age.

Children are exposed to arsenic in many of the same ways that adults are. Since arsenic is found in the soil, water, food, and air, children may take in arsenic in the air they breathe, the water they drink, and the food they eat. Since children tend to eat or drink less of a variety of foods and beverages than do adults, ingestion of contaminated food or juice or infant formula made

with arsenic-contaminated water may represent a significant source of exposure. In addition, since children often play in the soil and put their hands in their mouths and sometimes intentionally eat soil, ingestion of contaminated soil may be a more important source of arsenic exposure for children than for adults. In areas of the United States where natural levels of arsenic in the soil and water are high, or in areas in and around contaminated waste sites, exposure of children to arsenic through ingestion of soil and water may be significant. In addition, contact with adults who are wearing clothes contaminated with arsenic (e.g., with dust from copper- or lead-smelting factories, from wood-treating or pesticide application, or from arsenic-treated wood) could be a source of exposure. Because of the tendency of children to taste things that they find, accidental poisoning from ingestion of pesticides is also a possibility. Thus, although most of the exposure pathways for children are the same as those for adults, children may be at a higher risk of exposure because of normal hand-to-mouth activity.

Children who are exposed to inorganic arsenic may have many of the same effects as adults, including irritation of the stomach and intestines, blood vessel damage, skin changes, and reduced nerve function. Thus, all health effects observed in adults are of potential concern in children. There is also some evidence that suggests that long-term exposure to inorganic arsenic in children may result in lower IQ scores. We do not know if absorption of inorganic arsenic from the gut in children differs from adults. There is some evidence that exposure to arsenic in early life (including gestation and early childhood) may increase mortality in young adults.

There is some evidence that inhaled or ingested inorganic arsenic can injure pregnant women or their unborn babies, although the studies are not definitive. Studies in animals show that large doses of inorganic arsenic that cause illness in pregnant females can also cause low birth weight, fetal malformations, and even fetal death. Arsenic can cross the placenta and has been found in fetal tissues. Arsenic is found at low levels in breast milk.

In animals, exposure to organic arsenic compounds can cause low birth weight, fetal malformations, and fetal deaths. The dose levels that cause these effects also result in effects in the mothers.

You can find more information about how arsenic can affect children in Sections 3.7 and 6.6.

1.7 HOW CAN FAMILIES REDUCE THE RISK OF EXPOSURE TO ARSENIC?

If your doctor finds that you have been exposed to substantial amounts of arsenic, ask whether your children might also have been exposed. Your doctor might need to ask your state health department to investigate.

Many communities may have high levels of arsenic in their drinking water, particularly from private wells, because of contamination or as a result of the geology of the area. The north central region and the western region of the United States have the highest arsenic levels in surface water and groundwater sources, respectively. Wells used to provide water for drinking and cooking should be tested for arsenic. As of January 2006, EPA's Maximum Contaminant Level (MCL) for arsenic in drinking water is 10 ppb. If you have arsenic in your drinking water at levels higher that the EPA's MCL, an alternative source of water should be used for drinking and cooking should be considered.

If you use arsenic-treated wood in home projects, personal protection from exposure to arsenic-containing sawdust may be helpful in limiting exposure of family members. These measures may include dust masks, gloves, and protective clothing. Arsenic-treated wood should never be burned in open fires, or in stoves, residential boilers, or fire places, and should not be composted or used as mulch. EPA's Consumer Awareness Program (CAP) for CCA is a voluntary program established by the manufacturers of CCA products to inform consumers about the proper handling, use, and disposal of CCA-treated wood. You can find more information about this program in Section 6.5. Hand washing can reduce the potential exposure of children to arsenic after playing on play structures constructed with CCA-treated wood, since most of the arsenic on the children's hands was removed with water.

If you live in an area with a high level of arsenic in the water or soil, substituting cleaner sources of water and limiting contact with soil (for example, through use of a dense groundcover or thick lawn) would reduce family exposure to arsenic. By paying careful attention to dust and soil

control in the home (air filters, frequent cleaning), you can reduce family exposure to contaminated soil. Some children eat a lot of soil. You should prevent your children from eating soil. You should discourage your children from putting objects in their mouths. Make sure they wash their hands frequently and before eating. Discourage your children from putting their hands in their mouths or engaging in other hand-to-mouth activities. Since arsenic may be found in the home as a pesticide, household chemicals containing arsenic should be stored out of reach of young children to prevent accidental poisonings. Always store household chemicals in their original labeled containers; never store household chemicals in containers that children would find attractive to eat or drink from, such as old soda bottles. Keep your Poison Control Center's number by the phone.

It is sometimes possible to carry arsenic from work on your clothing, skin, hair, tools, or other objects removed from the workplace. This is particularly likely if you work in the fertilizer, pesticide, glass, or copper/lead smelting industries. You may contaminate your car, home, or other locations outside work where children might be exposed to arsenic. You should know about this possibility if you work with arsenic.

Your occupational health and safety officer at work can and should tell you whether chemicals you work with are dangerous and likely to be carried home on your clothes, body, or tools and whether you should be showering and changing clothes before you leave work, storing your street clothes in a separate area of the workplace, or laundering your work clothes at home separately from other clothes. Material safety data sheets (MSDS) for many chemicals used should be found at your place of work, as required by the Occupational Safety and Health Administration (OSHA) in the U.S. Department of Labor. MSDS information should include chemical names and hazardous ingredients, and important properties, such as fire and explosion data, potential health effects, how you get the chemical(s) in your body, how to properly handle the materials, and what to do in the case of emergencies. Your employer is legally responsible for providing a safe workplace and should freely answer your questions about hazardous chemicals. Your state OSHA-approved occupational safety and health program or OSHA can answer any further questions and help your employer identify and correct problems with hazardous substances. Your state OSHA-approved occupational safety and health program or

OSHA will listen to your formal complaints about workplace health hazards and inspect your workplace when necessary. Employees have a right to seek safety and health on the job without fear of punishment.

You can find more information about how arsenic can affect children in Sections 3.7 and 6.6.

1.8 IS THERE A MEDICAL TEST TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO ARSENIC?

Several sensitive and specific tests can measure arsenic in your blood, urine, hair, or fingernails, and these tests are often helpful in determining if you have been exposed to above-average levels of arsenic in the past. These tests are not usually performed in a doctor's office. They require sending the sample to a testing laboratory.

Measurement of arsenic in your urine is the most reliable means of detecting arsenic exposures that you experienced within the last several days. Most tests measure the total amount of arsenic present in your urine. This can sometimes be misleading, because the nonharmful forms of arsenic in fish and shellfish can give a high reading even if you have not been exposed to a toxic form of arsenic. For this reason, laboratories sometimes use a more complicated test to separate "fish arsenic" from other forms. Because most arsenic leaves your body within a few days, analysis of your urine cannot detect if you were exposed to arsenic in the past. Tests of your hair or fingernails can tell if you were exposed to high levels over the past 6–12 months, but these tests are not very useful in detecting low-level exposures. If high levels of arsenic are detected, this shows that you have been exposed, but unless more is known about when you were exposed and for how long, it is usually not possible to predict whether you will have any harmful health effects.

You can find more information on how arsenic can be measured in your hair, urine, nails, and other tissues in Chapters 3 and 7.

1.9 WHAT RECOMMENDATIONS HAS THE FEDERAL GOVERNMENT MADE TO PROTECT HUMAN HEALTH?

The federal government develops regulations and recommendations to protect public health. Regulations *can* be enforced by law. The EPA, the Occupational Safety and Health Administration (OSHA), and the Food and Drug Administration (FDA) are some federal agencies that develop regulations for toxic substances. Recommendations provide valuable guidelines to protect public health, but *cannot* be enforced by law. The Agency for Toxic Substances and Disease Registry (ATSDR) and the National Institute for Occupational Safety and Health (NIOSH) are two federal organizations that develop recommendations for toxic substances.

Regulations and recommendations can be expressed as "not-to-exceed" levels, that is, levels of a toxic substance in air, water, soil, or food that do not exceed a critical value that is usually based on levels that affect animals; they are then adjusted to levels that will help protect humans. Sometimes these not-to-exceed levels differ among federal organizations because they used different exposure times (an 8-hour workday or a 24-hour day), different animal studies, or other factors.

Recommendations and regulations are also updated periodically as more information becomes available. For the most current information, check with the federal agency or organization that provides it. Some regulations and recommendations for ARSENIC include the following:

The federal government has taken several steps to protect humans from arsenic. First, EPA has set limits on the amount of arsenic that industrial sources can release into the environment. Second, EPA has restricted or canceled many of the uses of arsenic in pesticides and is considering further restrictions. Third, in January 2001, the EPA lowered the limit for arsenic in drinking water from 50 to 10 ppb. Finally, OSHA has established a permissible exposure limit (PEL), 8-hour time-weighted average, of 10 $\mu g/m^3$ for airborne arsenic in various workplaces that use inorganic arsenic.

You can find more information on regulations and guidelines that apply to arsenic in Chapter 8.

1.10 WHERE CAN I GET MORE INFORMATION?

If you have any more questions or concerns, please contact your community or state health or environmental quality department, or contact ATSDR at the address and phone number below.

ATSDR can also tell you the location of occupational and environmental health clinics. These clinics specialize in recognizing, evaluating, and treating illnesses that result from exposure to hazardous substances.

Toxicological profiles are also available on-line at www.atsdr.cdc.gov and on CD-ROM. You may request a copy of the ATSDR ToxProfiles™ CD-ROM by calling the toll-free information and technical assistance number at 1-800-CDCINFO (1-800-232-4636), by e-mail at cdcinfo@cdc.gov, or by writing to:

Agency for Toxic Substances and Disease Registry
Division of Toxicology and Environmental Medicine
1600 Clifton Road NE
Mailstop F-32
Atlanta, GA 30333
Fax: 1-770-488-4178

Organizations for-profit may request copies of final Toxicological Profiles from the following:

National Technical Information Service (NTIS)
5285 Port Royal Road
Springfield, VA 22161
Phone: 1-800-553-6847 or 1-703-605-6000
Web site: http://www.ntis.gov/

2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO ARSENIC IN THE UNITED STATES

Arsenic is widely distributed in the Earth's crust, which contains ~3.4 ppm arsenic. In nature, arsenic is mostly found in minerals and only to a small extent in its elemental form. Arsenic is mainly obtained as a byproduct of the smelting of copper, lead, cobalt, and gold ores. Arsenic trioxide is the primary form in which arsenic is marketed and consumed. There has been no domestic production of arsenic since 1985. In 2003, the world's largest producer of arsenic compounds was China, followed by Chile and Peru.

In 2003, the United States was the world's largest consumer of arsenic. Production of wood preservatives, primarily copper chromated arsenate (CCA), $CrO_3 \bullet CuO \bullet As_2O_5$, accounted for >90% of domestic consumption of arsenic trioxide. In response to consumer concerns, U.S. manufacturers of arsenical wood preservative began a voluntary transition from CCA to other wood preservatives for certain residential wood products. This phase-out was completed on December 31, 2003; wood treated prior to this date could still be used and CCA-treated wood products continue to be used in industrial applications.

Other uses for arsenic compounds include the production of agricultural chemicals, as an alloying element in ammunition and solders, as an anti-friction additive to metals used for bearings, and to strengthen lead-acid storage battery grids. High-purity arsenic (99.9999%) is used by the electronics industry for gallium-arsenide semiconductors for telecommunications, solar cells, and space research. Various organic arsenicals are still used in the United States as herbicides and as antimicrobial additives for animal and poultry feed. However, the use of inorganic arsenic compounds in agriculture has virtually disappeared beginning around the 1960s. Arsenic trioxide and arsenic acid were used as a decolorizer and fining agent in the production of bottle glass and other glassware. Arsenic compounds also have a long history of use in medicine, and have shown a re-emergence of late with the recent introduction of arsenic trioxide treatment for acute promyelocytic leukemia.

The principal route of exposure to arsenic for the general population is likely to be the oral route, primarily in the food and in the drinking water. Dietary exposures to total arsenic were highly variable, with a mean of 50.6 μg/day (range of 1.01–1,081 μg/day) for females and 58.5 μg/day (range of 0.21–1,276 μg/day) for males. U.S. dietary intake of inorganic arsenic has been estimated to range from 1 to 20 μg/day, with grains and produce expected to be significant contributors to dietary inorganic arsenic

intake. Drinking water generally contains an average of 2 μg/L of arsenic, although 12% of water supplies from surface water sources in the North Central region of the country and 12% of supplies from groundwater sources in the Western region have levels exceeding 20 μg/L. Arsenic is also widely distributed in surface water, groundwater, and finished drinking water in the United States. Surveys of arsenic concentrations in rivers and lakes indicate that most values are below 10 μg/L, although individual samples may range up to 3,400 μg/L. Arsenic released to the land at hazardous waste sites is likely to be relatively immobile due to a high capacity for soil binding, particularly to iron and manganese oxides. Exposure to arsenic from other pathways is generally small, but may be significant for areas with high levels of arsenic contamination or in occupational settings. For a more complete discussion of possible exposures to arsenic, see Chapter 6 of the profile.

2.2 SUMMARY OF HEALTH EFFECTS

Arsenic is a potent toxicant that may exist in several oxidation states and in a number of inorganic and organic forms. Most cases of arsenic-induced toxicity in humans are due to exposure to inorganic arsenic, and there is an extensive database on the human health effects of the common arsenic oxides and oxyacids. Although there may be some differences in the potency of different chemical forms (e.g., arsenites tend to be somewhat more toxic than arsenates), these differences are usually minor. An exception would be arsine, which is highly toxic. However, because arsine and its methyl derivatives are gases or volatile liquids and are unlikely to be present at levels of concern at hazardous waste sites, health effect data for these compounds are not discussed in this document. Humans may be exposed to organic arsenicals (mainly methyl and phenyl derivatives of arsenic acid) that are used in agriculture and to organic arsenicals found in fish and shellfish (arsenobetaine and arsenocholine). Although the toxicity of organic arsenicals has not been as extensively investigated as inorganic arsenicals, there are sufficient animal data to evaluate the toxicity of methyl arsenates (e.g., monomethylarsonic acid [MMA] and dimethylarsinic acid [DMA]) and roxarsone. The so-called “fish arsenic” compounds (e.g., arsenobetaine) are not thought to be toxic and health effects data are not discussed in this document.

It is generally accepted that the arsenic-carbon bond is quite strong and most mammalian species do not have the capacity to break this bond; thus, inorganic arsenic is not formed during the metabolism of organic arsenicals. In most species, including humans, ingested (or exogenous) MMA(V) and DMA(V) undergo limited metabolism, do not readily enter the cell, and are primarily excreted unchanged in the urine. This is in contrast to inorganic arsenic, which undergoes sequential reduction and methylation reactions leading to the formation of MMA and DMA. Inorganic As(V) is readily reduced to inorganic

As(III), which is taken up by the cell. Within the cell (primarily in the liver), As(III) is methylated to form MMA(V), which is reduced to MMA(III); MMA(III) subsequently undergoes oxidative methylations to form DMA(V). DMA(V) is the primary excretion product in humans. Because inorganic and organic arsenicals exhibit distinct toxicokinetic characteristics, the health effects and MRLs are considered separately.

Inorganic Arsenicals. Exposures of humans near hazardous waste sites could involve inhalation of arsenic dusts in air, ingestion of arsenic in water, food, or soil, or dermal contact with contaminated soil or water. Increased risk of lung cancer, respiratory irritation, nausea, skin effects, and neurological effects have been reported following inhalation exposure. There are only a few quantitative data on noncancer effects in humans exposed to inorganic arsenic by the inhalation route. Animal data similarly identify effects on the respiratory system as the primary noncancer effect of inhaled inorganic arsenic compounds, although only a few studies are available. Only limited data on the effects of inhaled organic arsenic compounds in humans or animals are available; these studies are generally limited to high-dose, short-term exposures, which result in frank effects.

Relatively little information is available on effects due to direct dermal contact with inorganic arsenicals, but several studies indicate that the chief effect is local irritation and dermatitis, with little risk of other adverse effects.

The database for the oral toxicity of inorganic arsenic is extensive, containing a large number of studies of orally-exposed human populations. These studies have identified effects on virtually every organ or tissue evaluated, although some end points appear to be more sensitive than others. The available data from humans identify the skin as the most sensitive noncancer target following long-term oral arsenic exposure. Typical dermal effects include hyperkeratinization of the skin (especially on the palms and soles), formation of multiple hyperkeratinized corns or warts, and hyperpigmentation of the skin with interspersed spots of hypopigmentation. Oral exposure data from studies in humans indicate that these lesions typically begin to manifest at exposure levels of about 0.002–0.02 mg As/kg/day, but one study suggests that lesions may appear at even lower levels. At these exposure levels, peripheral vascular effects are also commonly noted, including cyanosis, gangrene, and, in Taiwanese populations, the condition known as “Blackfoot Disease.” Other reported cardiovascular effects of oral exposure to inorganic arsenic include increased incidences of high blood pressure and circulatory problems. The use of intravenous arsenic trioxide as therapy for acute promyelocytic leukemia has raised further concerns about the cardiovascular effects of arsenic, including alterations in cardiac QT interval and the

development of torsades de pointes. Decrements in lung function, assessed by spirometry, have been reported in subjects exposed to approximately 0.008–0.04 mg As/kg/day in the drinking water who exhibited skin lesions.

In addition to dermal, cardiovascular, and respiratory effects, oral exposure to inorganic arsenic may result in effects on other organ systems. Nausea, vomiting, and diarrhea are very common symptoms in humans following oral exposure to inorganic arsenicals, both after acute high-dose exposure and after repeated exposure to lower doses; these effects are likely due to a direct irritation of the gastrointestinal mucosa. Acute, high-dose exposure can lead to encephalopathy, with clinical signs such as confusion, hallucinations, impaired memory, and emotional lability, while long-term exposure to lower levels can lead to the development of peripheral neuropathy characterized by a numbness in the hands and feet that may progress to a painful "pins and needles" sensation. Recent studies also have reported neurobehavioral alterations in arsenic-exposed children.

Chronic exposure of humans to inorganic arsenic in the drinking water has been associated with excess incidence of miscarriages, stillbirths, preterm births, and infants with low birth weights. Animal data suggest that arsenic may cause changes to reproductive organs of both sexes, including decreased organ weight and increased inflammation of reproductive tissues, although these changes may be secondary effects. However, these changes do not result in a significant impact on reproductive ability. Animal studies of oral inorganic arsenic exposure have reported developmental effects, but generally only at concentrations that also resulted in maternal toxicity.

Arsenic is a known human carcinogen by both the inhalation and oral exposure routes. By the inhalation route, the primary tumor types are respiratory system cancers, although a few reports have noted increased incidence of tumors at other sites, including the liver, skin, and digestive tract. In humans exposed chronically by the oral route, skin tumors are the most common type of cancer. In addition to skin cancer, there are a number of case reports and epidemiological studies that indicate that ingestion of arsenic also increases the risk of internal tumors (mainly of bladder and lung, and to a lesser extent, liver, kidney, and prostate).

The Department of Health and Human Services (DHHS) has concluded that inorganic arsenic is known to be a human carcinogen. The International Agency for Research on Cancer (IARC) cites sufficient evidence of a relationship between exposure to arsenic and human cancer. The IARC classification of arsenic is Group 1. The EPA has determined that inorganic arsenic is a human carcinogen by the

inhalation and oral routes, and has assigned it the cancer classification, Group A. EPA has calculated an oral cancer slope factor of 1.5 $(mg/kg/day)^{-1}$ and a drinking water unit risk of $5x10^{-5}$ $(\mu g/L)^{-1}$ for inorganic arsenic based on human dose-response data. The inhalation unit risk for cancer is calculated to be 0.0043 $(\mu g/m^3)^{-1}$. The unit risk is the upper-bound excess lifetime cancer risk estimated to result from continuous exposure to an agent at a concentration of 1 μg/L in water or 1 $\mu g/m^3$ in air. EPA is currently revising the assessment for inorganic arsenic; a more detailed discussion of the uncertainties associated with human cancer risk levels for arsenic is presented in Section 3.2.2.7.

The following sections discuss significant effects resulting from exposure to inorganic arsenic in greater detail: dermal, cardiovascular, respiratory, gastrointestinal, neurological, and cancer. Additional information on these effects and on other effects is discussed in Section 3.2.

Dermal Effects. The most characteristic effect of long-term oral exposure to inorganic arsenic compounds is the development of skin lesions; these lesions are often used as diagnostic criteria for arsenicosis. The three lesions most often associated with chronic arsenicosis are hyperkeratinization of the skin (especially on the palms and soles), formation of multiple hyperkeratinized corns or warts, and hyperpigmentation of the skin with interspersed spots of hypopigmentation. Numerous studies of long-term, low-level exposure to inorganic arsenic in humans have reported the presence of these lesions. In general, they begin to manifest at chronic exposure levels >0.02 mg As/kg/day. Chronic oral studies of lower exposure levels, ranging from 0.0004 to 0.01 mg As/kg/day, have generally not reported dermal effects. However, in a study with detailed exposure assessment, all confirmed cases of skin lesions ingested water containing >100 μg/L arsenic (approximately 0.0037 mg As/kg/day) and the lowest known peak arsenic concentration ingested by a case was 0.115 μg/L (approximately 0.0043 mg As/kg/day). Another large study reported increased incidence of skin lesions associated with estimated doses of 0.0012 mg As/kg/day (0.023 mg As/L drinking water). The mechanism(s) by which inorganic arsenic causes dermal effects is not well-understood. Elucidating the mechanism of dermal effects has been particularly difficult because the dermal effects common in humans have not been seen in studies in animals.

Dermal effects have also been reported following inhalation exposures to inorganic arsenic, although they are not as diagnostic as for oral exposure. Several studies of arsenic-exposed workers have reported the development of dermatitis; exposure levels required to produce this condition are not well-established. Altered dermal pigmentation and hyperkeratosis have also been reported in studies of humans exposed to inorganic arsenic by inhalation, although exposure levels have varied considerably. Direct dermal contact

with inorganic arsenicals may cause irritation and contact dermatitis. Usually, the effects are mild (erythema and swelling), but may progress to papules, vesicles, or necrotic lesions in extreme cases; these conditions tend to heal without treatment if exposure ceases.

Cardiovascular Effects. A large number of studies in humans have reported cardiovascular effects following oral exposure to inorganic arsenic compounds. The cardiac effects of arsenic exposure are numerous, and include altered myocardial depolarization (prolonged QT interval, nonspecific ST segment changes), cardiac arrhythmias, and ischemic heart disease. These effects have been seen after acute and long-term exposure to inorganic arsenic in the environment, as well as side effects from intravenous therapy with arsenic trioxide for acute promyelocytic leukemia. Exposure levels for environmental exposures have not been well characterized, but intravenous doses for arsenic trioxide therapy are generally on the order of 0.15 mg As/kg/day.

Chronic exposure to inorganic arsenic has also been shown to lead to effects on the vascular system. The most dramatic of these effects is “Blackfoot Disease,” a disease characterized by a progressive loss of circulation in the hands and feet, leading ultimately to necrosis and gangrene. Blackfoot Disease is endemic in an area of Taiwan where average drinking water levels of arsenic range from 0.17 to 0.80 ppm, corresponding to doses of about 0.014–0.065 mg As/kg/day. The results of a another study suggested that individuals with a lower capacity to methylate inorganic arsenic to DMA have a higher risk of developing peripheral vascular disease in the Blackfoot Disease-hyperendemic area in Taiwan. Arsenic exposure in Taiwan has also been associated with an increased incidence of cerebrovascular and microvascular diseases and ischemic heart disease. While Blackfoot Disease itself has not been reported outside of Taiwan, other vascular effects are common in areas with high arsenic exposures, and include such severe effects as increases in the incidences of Raynaud's disease and of cyanosis of fingers and toes as well as hypertension, thickening and vascular occlusion of blood vessels, and other unspecified cardiovascular conditions. However, while the majority of human studies have reported cardiovascular effects following exposure to inorganic arsenic, some have found no such effects.

Changes in cardiac rhythm and in some vascular end points have also been reported in animal studies of inorganic arsenicals, but generally only at higher exposure levels and not to the degree of severity seen in humans.

Respiratory Effects. While case reports and small cohort studies have routinely reported an increase in respiratory symptoms of humans exposed occupationally to inorganic arsenic, dose-response data for

these symptoms are generally lacking. The only study that evaluated respiratory effects (changes in chest x-ray or respiratory performance) and reported an exposure estimate did not report significant changes at an exposure level of 0.613 mg As/m^3. Exposed workers often report irritation of the mucous membranes of the nose and throat, which may lead to laryngitis, bronchitis, or rhinitis. Increased mortality due to respiratory disease has been reported in some cohort mortality studies of arsenic-exposed workers, but no conclusive evidence of an association of these diseases with arsenic exposure has been presented. It is not known whether respiratory effects following inhaled inorganic arsenic compounds are due to a direct effect of arsenic on respiratory tissues, general effects of foreign material in the lungs, or an effect of arsenic on the pulmonary vasculature. Similar responses, including rales, labored breathing, and respiratory hyperplasia, have been noted in animal studies of inhaled or instilled inorganic arsenic compounds.

Respiratory effects have also been reported following oral exposure of humans to inorganic arsenic. Acute oral exposure to ≥8 mg As/kg may result in serious respiratory effects, including respiratory distress, hemorrhagic bronchitis, and pulmonary edema; however, it is not clear whether these are primary effects or are the result of damage to the pulmonary vascular system. In general, respiratory effects have not been widely associated with long-term oral exposure to low arsenic doses. However, some studies have reported minor respiratory symptoms, such as cough, sputum, rhinorrhea, and sore throat, in people with repeated oral exposure to 0.03–0.05 mg As/kg/day. More serious respiratory effects, such as bronchitis and sequelae (bronchiectasis, bronchopneumonia) have been observed in patients chronically exposed to arsenic and at autopsy in some chronic poisoning cases. There are few animal data reporting respiratory effects of oral exposure to inorganic arsenic, and those studies generally found effects only at very high dose levels.

Gastrointestinal Effects. Both short-term and chronic oral exposures to inorganic arsenicals have been reported to result in irritant effects on gastrointestinal tissues. Numerous studies of acute, high-dose exposure to inorganic arsenicals have reported nausea, vomiting, diarrhea, and abdominal pain, although specific dose levels associated with the onset of these symptoms have not been identified. Chronic oral exposure to 0.01 mg As/kg/day generally results in similar reported symptoms. For both acute and chronic exposures, the gastrointestinal effects generally diminish or resolve with cessation of exposure. Similar gastrointestinal effects have been reported after occupational exposures to inorganic arsenicals, although it is not known if these effects were due to absorption of arsenic from the respiratory tract or from mucociliary clearance resulting in eventual oral exposure.

Neurological Effects. A common effect following both oral and inhalation exposure to inorganic is the development of peripheral neuropathy. Following occupational exposure to inorganic arsenic in pesticide plants or smelters, exposed workers have shown increased incidence of neurological changes, including altered nerve conduction velocities. One study reported that these effects were seen after 28 years of exposure to 0.31 mg As/m^3. In another study, signs and symptoms of sensory and motor polyneuropathy on both upper and lower extremities were reported in workers at a power station in Slovakia. The average length of exposure was 22.3 years (standard deviation [SD] ±8.4 years) and the average arsenic exposure in inhaled air ranged from 4.6 to 142.7 μg/m^3.

Following high-dose (>2 mg As/kg/day) acute oral exposures to inorganic arsenicals in humans, reported effects include headache, lethargy, mental confusion, hallucination, seizures, and coma. Following longer-term exposure to 0.03–0.1 mg As/kg/day, peripheral neuropathy, characterized initially by numbness of the hands and feet and a “pins and needles” sensation and progressing to muscle weakness, wrist-drop and/or ankle-drop, diminished sensitivity, and altered reflex action. Histological features of the neuropathy include a dying-back axonopathy and demyelination. Following removal from exposure, the neuropathy is only partially reversible and what recovery does occur is generally slow. Reports of neurological effects at lower arsenic levels (0.004–0.006 mg As/kg/day) have been inconsistent, with some human studies reporting fatigue, headache, depression, dizziness, insomnia, nightmare, and numbness while others reported no neurological effects. Some studies also have reported that exposure to arsenic may be associated with intellectual deficits in children. Neurological effects have also been reported in oral studies of arsenic toxicity in animals, although these were generally performed at higher doses (0.4–26.6 mg As/kg/day) than has been reported in exposed human populations. The mechanism(s) of arsenic-induced neurological changes has not been determined.

Cancer. There is clear evidence from studies in humans that exposure to inorganic arsenic by either the inhalation or oral routes increases the risk of cancer. Numerous studies of copper smelters or miners exposed to arsenic trioxide have reported an increased risk of lung cancer. Increased incidence of lung cancer has also been observed at chemical plants where exposure was primarily to arsenate. Other studies suggest that residents living near smelters or arsenical chemical plants may have increased risk of lung cancer, although the reported increases are small and are not clearly detectable in all cases. In general, studies reporting long-term exposure to 0.07 mg As/m^3 or greater have shown an increased incidence of lung cancer, while at lower exposure levels, the association has been less clear or not present.

There is convincing evidence from a large number of epidemiological studies and case reports that ingestion of inorganic arsenic increases the risk of developing skin cancer. The most common tumors seen are squamous cell carcinomas, which may develop from the hyperkeratotic warts or corns commonly seen as a dermal effect of oral inorganic arsenic exposure. Early studies of populations within the United States did not suggest an increased risk of cancer from oral inorganic arsenic exposure. Later studies have found suggestive evidence that the possibility of arsenic-induced skin cancers cannot be discounted based on an association between toenail arsenic levels and incidence of skin cancer.

There is increasing evidence that long-term exposure to arsenic can result in the development of bladder cancer, with transitional cell cancers being the most prevalent. While studies have noted statistical dose-response trends in arsenic-induced bladder cancers, reliable quantitative assessments of dose-response relationships have not been presented. Several studies have also shown that chronic oral exposure to arsenic results in the development of respiratory tumors, making lung cancer an established cause of death from exposure to arsenic in drinking water. Exposure levels in studies evaluating respiratory and bladder cancers have been comparable to those in studies evaluating skin tumors. Studies of U.S. populations have not identified an increased risk of bladder or respiratory tumors following oral exposure to inorganic arsenic.

Animal studies of both inhalation and oral exposure to inorganic arsenicals have not resulted in increased incidence of cancer formation in adult animals. However, a series of studies have shown that inorganic arsenic can induce cancer in the offspring from mice exposed to arsenic during gestation (transplacental carcinogen) and acts as a co-carcinogen with UV light and polycyclic aromatic hydrocarbons (PAHs).

Organic Arsenicals. Humans may be exposed to organic arsenicals via inhalation of dusts, ingestion of organic arsenic in water, food, soil, or dermal contact with contaminated soil, water or plants following pesticide application. There are limited data on the toxicity of organic arsenicals following inhalation exposure in humans and animals and these data do not allow for identification of critical effects. Keratosis was observed in workers exposed to 0.065 mg/m^3 arsanilic acid (i.e., 4-aminophenyl arsenic acid); no alterations in gastrointestinal symptoms or hematological alterations were observed. In animals, very high concentrations (>3,000 mg/m^3) of DMA results in respiratory distress, diarrhea, and erythematous lesions on the feet and ears. No adverse effects were observed in rats exposed to DMA concentrations as high as 100 mg DMA/m^3 for 95 days.

Similarly, the available dermal toxicity data do not allow for identification of critical effects. Contact dermatitis was observed in workers applying DMA (and its sodium salt) and mild dermal irritation was observed in a Draize test in rabbits (adverse effect level not reported). Intermediate duration (21 days) exposure studies in rabbits did not result in systemic toxicity or skin irritation following 5 day/week exposure to 1,000 mg/kg/day MMA or DMA.

The preponderance of toxicity data for organic arsenicals involves oral exposure. Human data are limited to three case reports of individuals intentionally ingesting pesticides containing organic arsenicals. Gastrointestinal irritation (vomiting, nausea, and diarrhea) were consistently reported in these cases. Animal data has primarily focused on the toxicity of MMA, DMA, and roxarsone; these data suggest that the targets of toxicity may differ between the compounds.

MMA. The gastrointestinal tract appears to be the most sensitive target of toxicity for MMA. Diarrhea and tissue damage in the large intestine have been reported in several animal species following dietary, gavage, and capsule exposure. For diarrhea, both the time of onset and incidence appear to be dose-related. In rats, diarrhea was observed in 100% of females exposed to 98.5 mg MMA/kg/day, 55% of females exposed to 33.9 mg MMA/kg/day, and 5.1% of females exposed to 3.9 mg MMA/kg/day. The increased incidence of diarrhea was observed after 3 weeks of exposure to 98.5 mg MMA/kg/day, 4 weeks at 33.9 mg MMA/kg/day, and 18 months at 3.9 mg MMA/kg/day. Histological damage consisting of squamous metaplasia of the epithelial columnar absorptive cells in the cecum, colon, and rectum was observed in rats and mice chronically exposed to 72.4 or 67.1 mg MMA/kg/day, respectively. Hemorrhagic, necrotic, ulcerated, or perforated mucosa were also observed in the large intestine of rats exposed to 67.1 mg MMA/kg/day for 2 years. In rats, the damage to the large intestine resulted in intestinal contents leaking into the abdominal cavity and the development of peritonitis. The available data provide suggestive evidence that there may be some species differences in the sensitivity to gastrointestinal damage; however, some of these differences may be due to the route of administration. The lowest adverse effect levels, regardless of duration of exposure, for gastrointestinal effects in rats, mice, rabbits, and dogs are 25.7, 67.1, 12, and 2 mg MMA/kg/day, respectively; the no adverse effect levels in rats and mice (NOAELs were not identified in rabbits and dogs) were 3.0 and 24.9 mg MMA/kg/day. However, the rabbit and dog studies involved bolus administration (gavage and capsule administration), which may have increased sensitivity; the rat and mouse studies involved dietary exposure.

The kidney also appears to be a sensitive target in rats and mice chronically exposed to MMA. An increase in the severity of progressive glomerulonephropathy was observed in female rats exposed to 33.9 mg MMA/kg/day for 2 years and an increase in the incidence of progressive glomerulonephropathy was observed in male mice exposed to 6.0 mg MMA/kg/day for 2 years. Other adverse effects that have been observed in animals exposed to MMA include hypertrophy of the thyroid follicular cells in rats exposed to 33.9 mg MMA/kg/day in the diet for 2 years, reproductive toxicity, and developmental toxicity. Decreases in pregnancy rate and male fertility index were observed in F_0 and F_1 rats exposed to 76 mg MMA/kg/day for 14 weeks prior to mating and during the mating period; the findings were not significantly different than control values but were considered treatment-related because they were outside the range found in historical controls. This study also reported a decrease in pup survival in the F_1 and F_2 offspring of rats exposed to 76 mg MMA/kg/day; as with the reproductive effects, the incidence was not statistically different from controls but was considered biologically significant because survival in the MMA pups was outside the range found in historical controls. Another study reported impaired fetal growth (decreases in fetal weights and incomplete ossification) and minor skeletal defects (an increase in the number of fetuses with supernumerary thoracic ribs and eight lumbar vertebrae) in rat and rabbit fetuses exposed to 500 or 12mg MMA/kg/day, respectively; maternal toxicity was also observed at these dose levels and the effects may be secondary to maternal stress rather than a direct effect on the developing organisms. A 2-year bioassay did not result in significant increases in the incidence of neoplastic lesions in rats and mice exposed to doses as high as 72.4 and 67.1 mg MMA/kg/day, respectively.

DMA. The most sensitive targets of DMA toxicity in rats are the urinary bladder and kidneys. In the bladder, the effects progress from cytotoxicity to cellular necrosis to regenerative proliferation and hyperplasia. At dietary doses of 11 mg DMA/kg/day, cytotoxicity is observed as early as 6 hours after exposure initiation and cellular proliferation (as evident by increased BrdU labeling) was observed after 2 weeks of exposure. After 10 weeks of exposure, necrosis and hyperplasia were also observed. The lowest adverse effect levels for urinary bladder effects following intermediate or chronic duration exposure were 5 mg DMA/kg/day for evidence of regenerative proliferation and 3.1 mg DMA/kg/day for vacuolar degeneration of urothelium and hyperplasia. Vacuolization of the superficial cells of the urothelium was observed in mice exposed to 7.8 mg DMA/kg/day and higher for 2 years. However, unlike the vacuolar degeneration observed in rats, the vacuolization observed in mice was not associated with cytotoxicity, necrosis, inflammation, or hyperplasia.

Kidney damage characterized by increased urinary calcium levels, calcification, nephrocalcinosis, and necrosis of the renal papillae have been observed in rats following intermediate- or chronic-duration exposure. Increases in urine calcium levels and corticomedullary junction calcification were observed in rats exposed to 5 or 10 mg DMA/kg/day for 10 weeks and cortical degeneration and necrosis were observed in rats exposed to 57 mg DMA/kg/day for 4 weeks. Chronic-duration exposure to 3.1 mg DMA/kg/day resulted in an increased incidence of nephrocalcinosis and necrosis of the renal papillae in rats; these lesions are typical in aged rats, although DMA exposure appeared to exacerbate them. An exacerbation of age-related kidney lesion (progressive glomerulonephropathy and nephrocalcinosis) has also been observed in male mice exposed to 37 or 94 mg DMA/kg/day, respectively, for 2 years. A consistent finding in intermediate and chronic rat studies is an increase in urine volume, which corresponds to an increase in water consumption; the toxicological significance of this finding is not known. The observed decreases in electrolyte levels and specific gravity are likely due to the higher urine volume.

Although gastrointestinal effects have been observed in animals exposed to DMA, it does not appear to be as sensitive a target compared to MMA. Diarrhea has been observed in rats exposed to a lethal dose of 190 mg DMA/kg/day for 4 weeks and in dogs administered via 16 mg DMA/kg/day. No gastrointestinal effects were observed in rats or mice chronically exposed to 7.8 or 94 mg DMA/kg/day.

Other adverse effects that have been observed in animals exposed to organic arsenicals include hypertrophy of thyroid follicular cells in rats exposed to 4.0 mg DMA/kg/day in the diet for 13 weeks and 7.8 mg DMA/kg/day in the diet for 2 years and developmental effects in rats and mice. Decreases in fetal growth and delays in ossification have been observed in rat fetuses exposed to ≥36 mg DMA/kg/day; these alterations typically occur at doses associated with decreases in maternal weight gain. Other developmental effects that have been reported include an increase in the incidences of irregular palatine rugae in rats exposed to 30 mg DMA/kg/day, diaphragmatic hernia in rats exposed to 36 mg DMA/kg/day, and cleft palate in mice exposed to 400 mg DMA/kg/day. No developmental effects were observed in rabbits exposed to 12 mg DMA/kg/day.

The available data provide strong evidence that DMA is carcinogenic in rats. A 2-year exposure to DMA resulted in significant increases in the incidence of neoplastic urinary bladder tumors in rats exposed to 7.8 mg DMA/kg/day in the diet or 3.4 mg DMA/kg/day in drinking water. No increases in neoplastic tumors were observed in mice exposed to doses as high as 94 mg DMA/kg/day for 2 years; however, a

50-week exposure to 10.4 mg DMA/kg/day did result in an increased incidence of lung tumors in A/J mice.

The available data for DMA suggest that there are species differences in terms of the critical effects and sensitivity. In rats, the urinary bladder and kidneys are the most sensitive targets with effects occurring at 5 mg DMA/kg/day following intermediate-duration exposure and 3.1 mg DMA/kg/day following chronic-duration exposure. Although the urinary bladder and kidneys are also sensitive targets in mice with LOAELs of 7.8 and 37 mg DMA/kg/day, respectively, following chronic exposure, the effects are not associated with cytotoxicity or elevated urine calcium levels. In dogs, the most sensitive effect is gastrointestinal tract irritation (diarrhea), which occurs at 16 mg DMA/kg/day.

There is concern that the rat may not be a good model to predict the human risk associated with organic arsenic exposure due to the unique toxicokinetic properties of DMA in rats. In humans and most animal species, DMA is rapidly eliminated from the body; >90% of the dose is excreted 2–3 days after dosing. In contrast, DMA is slowly eliminated in rats. One study estimated that 45% of an initial oral DMA dose was eliminated with a half-time of 13 hours; the remaining 55% of the dose DMA dose had an elimination half-time of 50 days. In rats, DMA has a strong affinity for hemoglobin resulting in an accumulation of DMA in erythrocytes. Species differences in DMA metabolism have also been found. In particular, DMA undergoes further methylation to trimethylarsine oxide (TMAO) in rats. In most animal species, almost the entire oral DMA dose is excreted in the urine unchanged; however, in rats, about half of the dose is excreted in the urine as DMA and the other half as TMAO. During the metabolism of DMA to TMAO, DMA(III) is formed as a metabolic intermediate. The formation of this highly reactive intermediate and the excretion of small amounts of DMA(III) in urine may damage the urinary bladder.

There are limited data on the mode of action of DMA for most end points. Recently, there has been considerable research on the mode of action for the development of neoplastic urinary bladder tumors in rats. Although the mechanisms have not been fully elucidated, it has been proposed that the mode of action involves cytotoxicity leading to necrosis and subsequent regeneration of the urinary bladder urothelium. There is strong evidence to suggest that DMA(III) is the causative agent for the urothelial cytotoxicity. The strongest evidence comes from the finding that urinary concentrations of DMA(III) measured in rats exhibiting urothelial cytotoxicity are equivalent to DMA(III) concentrations that are cytotoxic to urothelial cells *in vitro*. Urothelial cytotoxicity, regenerative urothelial proliferation, and urothelial tumors have not been detected in other animal species. Other animal species, including

humans, only metabolize a small percentage of ingested DMA to TMAO; thus, much lower levels of DMA(III) are produced, suggesting that rats may be very sensitive to toxicity of DMA and therefore are not an appropriate model for human risk assessment.

Roxarsone. The available data on the toxicity of roxarsone suggest that following bolus administration, the gastrointestinal tract, kidney, and nervous system are sensitive end points of roxarsone toxicity. Vomiting and gastrointestinal hemorrhage were observed in dogs receiving a single capsulized dose of 50 mg/kg roxarone; no gastrointestinal effects were observed in rats or mice administered 4 or 42 mg/kg/day roxarsone for 2 years. Kidney effects included increases in kidney weight, minimal tubular epithelial cell degeneration, and focal mineralization in rats exposed to 32 mg roxarsone/kg/day for 13 weeks; no kidney effects were observed at 16 mg/kg/day or in mice exposed to doses as high as 136 mg/kg/day for 13 weeks or 43 mg/kg/day for 2 years. Hyperexcitability, ataxia, and/or trembling were observed in rats exposed to 20 mg/kg/day for 13 weeks or 64 mg/kg/day for 13 weeks. A 14-day study in rats reported slight inactivity in rats exposed to 32 mg/kg/day, but this was not observed in longer-term studies. Neurological effects were observed in mice exposed to doses as high as 136 mg/kg/day for 13 weeks or 43 mg/kg/day for 2 years, although a slight decrease in activity at 42 mg/kg/day was reported in a 14 day study. Pigs appear to be especially sensitive to the neurotoxicity of roxarsone. Muscle tremors have been observed at doses of ≥6.3 mg roxarsone/kg/day and myelin degeneration in the spinal cord was noted at 6.3 mg/kg/day. Both the clinical signs of neuropathy and the myelin degeneration followed a time-related pattern. Mild lethargy and ataxia were observed 7 days after exposure initiation, exercise-induced muscle tremors and clonic seizures were observed at day 11, paraparesis was observed at day 22, and paraplegia was observed at day 33. At day 11, equivocal lesions were observed in the cervical spinal cord, and the severity of these lesions increased with time; myelin degeneration was observed in the peripheral nerves and optic nerve starting at day 32 (2 days after exposure termination). Equivocal evidence of carcinogenicity (a slight increase in the incidence of pancreatic tumors) was found in male rats chronically exposed to roxarsone; no increases in neoplastic tumors were observed in female rats or male and female mice.

2.3 MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for arsenic. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive

health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990i), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

Inorganic Arsenicals

Inhalation MRLs. No inhalation MRLs were derived for inorganic arsenic. Human data suggest that dermal or respiratory effects may be the most prevalent (Lagerkvist et al. 1986; Mohamed 1998; Perry et al. 1948); respiratory or immunological effects appeared to be the most common following inhalation exposure to inorganic arsenic in animals (Aranyi et al. 1985; Holson et al. 1999). Adequate human studies evaluating dose-response relationships for noncancer end points were not located for inorganic arsenic, and animal data on the health effects of inorganic arsenic following inhalation exposure are limited to studies that did not evaluate a suitable range of health effects. Lacking suitable studies upon which to base the MRLs, no inhalation MRLs were derived for inorganic arsenic.

Oral MRLs

- An MRL of 0.005 mg As/kg/day has been derived for acute-duration (14 days or less) oral exposure to inorganic arsenic.

Mizuta et al. (1956) summarized findings from 220 poisoning cases associated with an episode of arsenic contamination of soy sauce in Japan. The soy sauce was contaminated with approximately 0.1 mg As/mL, probably as calcium arsenate. Arsenic intake in the cases was estimated by the researchers to be 3 mg/day (0.05 mg/kg/day, assuming 55 kg average body weight for this Asian population). The duration of exposure was 2–3 weeks in most cases. The primary symptoms were edema of the face, and gastrointestinal and upper respiratory symptoms initially, followed by skin lesions and neuropathy in

some patients. Other effects included mild anemia and leukopenia, mild degenerative liver lesions and hepatic dysfunction, abnormal electrocardiogram, and ocular lesions. For derivation of the acute oral MRL, facial edema and gastrointestinal symptoms (nausea, vomiting, diarrhea), which were characteristic of the initial poisoning and then subsided, were considered to be the critical effects. The MRL of 0.005 mg As/kg/day was calculated by applying an uncertainty factor of 10 (10 for use of a lowest-observed-adverse-effect level (LOAEL) and 1 for human variability) to the LOAEL of 0.05 mg As/kg/day (see Appendix A for MRL worksheets).

An intermediate-duration oral MRL for inorganic arsenic was not derived due to inadequacy of the database. The lowest LOAEL identified in a limited number of intermediate-duration human studies available was 0.05 mg As/kg/day in a study by Mizuta et al. (1956) (summarized above). While this study was considered appropriate to derive an acute-duration oral MRL for inorganic arsenic, there is considerable uncertainty regarding what the effects and severity might be beyond the relatively short 2–3 weeks of exposure that most subjects experienced. There are numerous studies in animals dosed for intermediate durations, but as indicated in Section 3.5.3, animals are not appropriate models for effects of inorganic arsenic in humans.

- An MRL of 0.0003 mg As/kg/day has been derived for chronic-duration (365 days or more) oral exposure to inorganic arsenic.

Tseng et al. (1968) and Tseng (1977) investigated the incidence of Blackfoot Disease and dermal lesions (hyperkeratosis and hyperpigmentation) in a large number of poor farmers (both male and female) exposed to high levels of arsenic in well water in Taiwan. A control group consisting of 17,000 people, including one group in which arsenic exposure was “undetermined” and which included those villages where arsenic-contaminated wells were no longer used or the level could not be classified, and a control population of 7,500 people who consumed water from wells almost free of arsenic (0.001–0.017 ppm) was also examined. The authors stated that the incidence of dermal lesions increased with dose, but individual doses were not provided. However, incidence data were provided based on stratification of the exposed population into low (<300 μg/L), medium (300–600 μg/L), or high (>600 μg/L) exposure levels. Doses were calculated from group mean arsenic concentrations in well water, assuming the intake parameters described by IRIS (IRIS 2007). Accordingly, the control, low-, medium-, and high-exposure levels correspond to doses of 0.0008, 0.014, 0.038, and 0.065 mg As/kg/day, respectively. The no-observed-adverse-effect level (NOAEL) identified by Tseng (1977) (0.0008 mg As/kg/day) was limited by the fact that the majority of the population was <20 years of age and the incidence of skin lesions increased as a function of age, and because the estimates of water intake and dietary arsenic intake are

highly uncertain. Schoof et al. (1998) estimated that dietary intakes of arsenic from rice and yams may have been 15–211 μg/day (mean=61 μg/day), based on arsenic analyses of foods collected in Taiwan in 1993–1995. Use of the 50 μg/day estimate would result in an approximate doubling of the NOAEL (0.0016 mg/kg/day) (see Appendix A for MRL worksheets). The MRL was derived by applying an uncertainty factor of 3 (for human variability) to the NOAEL of 0.0008 mg/kg/day.

The MRL is supported by a large number of well-conducted epidemiological studies that identify reliable NOAELs and LOAELs for dermal effects. EPA (1981b) identified a NOAEL of 0.006–0.007 mg As/kg/day for dermal lesions in several small populations in Utah. Harrington et al. (1978) identified a NOAEL of 0.003 mg As/kg/day for dermal effects in a small population in Alaska. Guha Mazumder et al. (1988) identified a NOAEL of 0.009 mg As/kg/day and a LOAEL of 0.006 mg As/kg/day for pigmentation changes and hyperkeratosis in a small population in India. Haque et al. (2003) identified a LOAEL of 0.002 mg As/kg/day for hyperpigmentation and hyperkeratosis in a case-control study in India. Cebrián et al. (1983) identified a NOAEL of 0.0004 mg As/kg/day and a LOAEL of 0.022 mg As/kg/day in two regions in Mexico. Borgoño and Greiber (1972) and Zaldívar (1974) identified a LOAEL of 0.02 mg As/kg/day for abnormal skin pigmentation in patients in Chile, and Borgoño et al. (1980) identified a LOAEL of 0.01 mg As/kg/day for the same effect in school children in Chile. Valentine et al. (1985) reported a NOAEL of 0.02 mg As/kg/day for dermal effects in several small populations in California. Collectively, these studies indicate that the threshold dose for hyper-pigmentation and hyperkeratosis is approximately 0.002 mg As/kg/day. While many of these studies also identified effects on other end points at these exposure levels, including effects on gastrointestinal (Borgoño and Greiber 1972; Cebrián et al. 1983; Guha Mazumder et al. 1988; Zaldívar 1974), cardiovascular (Tseng et al. 1995, 1996), hepatic (Hernández-Zavala et al. 1998), and neurological end points (Guha Mazumder et al. 1988; Lianfang and Jianzhong 1994; Tsai et al. 2003), the overall database for dermal effects is considerably stronger than for effects on other end points.

Organic Arsenicals

Inhalation MRLs. No inhalation MRLs were derived for organic arsenic. Human data are limited to an occupational exposure study of workers exposed to 0.065 mg/m^3 ansanilic acid (Watrous and McCaughey 1945). The exposed workers more frequently complained of keratosis than nonexposed workers. A limited number of animal studies have examined the toxicity of organic arsenicals following inhalation exposure. Respiratory distress and diarrhea were observed in rats and mice exposed to high concentrations of MMA and DMA (Stevens et al. 1979); at lower concentrations (1,540–3,150 mg DMA/m^3), respiratory irritation, as evidenced by a decrease in respiration rate, was observed in animals

exposed to MMA or DMA (Stevens et al. 1979). The acute-duration studies do not clearly identify the most sensitive targets of inorganic arsenical toxicity; the available studies are of limited scope and none included a comprehensive histological examination.

One study examined the toxicity of DMA in rats following intermediate-duration exposure. This study (Whitman 1994) found an increase in intracytoplasmic eosinophilic globules in the nasal turbinates of rats exposed to 34 or 100 mg/m^3 DMA 6 hours/day, 5 days/week for 67–68 exposures; no other adverse effects were observed in this comprehensive study. As discussed in greater detail in the oral MRL section, the toxicokinetic properties of DMA in rats differ from other species and rats do not appear to be a good model for human exposure. The half-time of DMA in the body is much longer in rats compared to other species, including humans, and DMA is more extensively methylated in rats. In the absence of data to determine whether the observed effect is due to a direct interaction of DMA, derivation of an intermediate-duration MRL using rat data is not recommended at this time.

No studies examined the chronic toxicity of organic arsenicals precluding the derivation of a chronic-duration inhalation MRL.

Oral MRLs

MMA. A limited number of animal studies have examined the acute oral toxicity of MMA. These studies consisted of LD_{50} studies in rats (Gur and Nyska 1990), mice (Kaise et al. 1989), and rabbits (Jaghabir et al. 1988) and developmental toxicity studies in rats (Irvine et al. 2006) and rabbits (Irvine et al. 2006); all studies administered MMA via gavage. Adverse effects reported in the LD_{50} studies included diarrhea in rats at 2,030 mg monosodium methane arsonate (MSMA)/kg (Gur and Nyska 1990), mice at 2,200 mg MMA/kg (Kaise et al. 1989) and rabbits at 60 mg MSMA/kg (Jaghabir et al. 1988) and respiratory arrest in mice at 1,800 mg MMA/kg/day (Kaise et al. 1989). These doses were at or near the LD_{50} levels of 2,449 mg MSMA/kg, 1,800 mg MMA/kg, 100 mg MSMA/kg for the rats, mice, and rabbits, respectively. In the developmental toxicity studies (Irvine et al. 2006), maternal effects included decreases in maternal body weight gain in rats (17% less than controls) and rabbits (70% less than controls) receiving gavage doses of 100 and 12 mg MMA/kg/day, respectively, and loose feces/diarrhea in rabbit does administered 12 mg MMA/kg/day. The NOAELs for maternal effects were 10 and 7 mg MMA/kg/day in the rats and rabbits, respectively. Minor developmental effects (decreased fetal weight, incomplete ossification, and supernumerary ribs) were also observed at the maternally toxic doses in the rats and rabbits (Irvine et al. 2006); these effects were probably secondary to the maternal stress. These

data, coupled with the results of longer-term studies (Arnold et al. 2003; Waner and Nyska 1988), suggest that the gastrointestinal tract is a sensitive target of MMA toxicity. The rabbit developmental toxicity study (Irvine et al. 2006) identified the lowest LOAEL (12 mg MMA/kg/day) for gastrointestinal irritation. However, this study is not suitable for the derivation of an acute-duration oral MRL for MMA because the MMA was administered via bolus doses. It is likely that the observed gastrointestinal effect is a concentration-dependent effect; thus, at a given dose level, effects are more likely to occur following bolus administration. A marked decrease in body weight gain was also observed at this dose level.

- An MRL of 0.1 mg MMA/kg/day has been derived for intermediate-duration (15–364 days) oral exposure to MMA.

Three studies have examined the intermediate-duration toxicity of MMA; two of these are chronic-duration studies reporting diarrhea and decreases in body weight gain after MMA exposure for <1 year. Diarrhea was observed in rats exposed to 30.2 mg MMA/kg/day in the diet (Arnold et al. 2003) and in dogs exposed via a capsule to 2 mg MMA/kg/day (Waner and Nyska 1988). Decreases in body weight were observed at the next highest doses, 106.9 mg MMA/kg/day in rats and 8 mg MMA/kg/day in dogs. In the rat study (Arnold et al. 2003), diarrhea was observed in 16.7 and 40% of the males and females, respectively, exposed to 30.2/35.9 mg MMA/kg/day during the first 52 weeks of the study; diarrhea first occurred after 4 weeks of exposure. At the highest dose level (106.9 mg MMA/kg/day), diarrhea was observed in all exposed male and female rats. In dogs, the increased incidence of diarrhea first occurred during weeks 25–28; at the highest dose tested in the study (35 mg MMA/kg/day), vomiting was also observed. A NOAEL of 3.5 mg MMA/kg/day was identified in the rat study; a NOAEL was not identified in the dog study. The remaining study in the intermediate-duration database is a 2-generation study that reported reproductive (decreased pregnancy rate and male fertility index in F_0 and F_1 generations) and developmental (decreased pup survival in F_1 and F_2 generation) effects in rats exposed to 76 mg MMA/kg/day in the diet (Schroeder 1994). The lowest LOAEL identified in the intermediate-duration database is 2 mg MMA/kg/day for diarrhea in dogs (Waner and Nyska 1988). Although dogs appear to be more sensitive to the gastrointestinal effects of MMA, a direct comparison of the two studies is not possible due to the difference in the routes of exposure. It is possible that the bolus administration of MMA, in the form of a capsule, resulted in increased sensitivity of the dogs. Because the most likely route of exposure for humans would be ingestion and the critical effect appears to be irritation of the gastrointestinal tract, studies involving bolus administration (gavage or capsule) were not considered for derivation of oral MRLs. The Arnold et al. (2003) and Schroeder (1994) studies were considered as the basis for an intermediate-duration MRL. Of these two studies, Arnold et al. (2003) identified the lowest

LOAEL, 30.2 mg MMA/kg/day, for gastrointestinal effects and was selected as the principal study for the intermediate-duration oral MRL.

Arnold et al. (2003) exposed groups of 60 male and 60 female Fischer 344 rats to 0, 50, 400, or 1,300 ppm MMA in the diet for 104 weeks. Using the average doses for weeks 1–50 reported in an unpublished version of this study (Crown et al. 1990), doses of 0, 3.5, 30.2, and 106.9 mg MMA/kg/day and 0, 4.2, 35.9, and 123.3 mg MMA/kg/day were calculated for males and females, respectively. Body weights, food consumption, and water intake were monitored regularly. Blood was taken at 3, 6, and 12 months for clinical chemistry measurements, and urine samples were collected at the same interval. Mortality was increased in high-dose males and females during the first 52 weeks of the study. Body weights were decreased in the mid- and high-dose groups of both sexes; however, at 51 weeks, only the body weight for the high-dose males was <10% of the control weight (14.5%). Food and water consumption was increased in the mid- and high-dose groups. Diarrhea was observed in 100% of the high-dose males and females and in 16.7 and 40% of the mid-dose males and females during the first 52 weeks of exposure. Diarrhea first occurred after 3 weeks of exposure to the high dose and 4 weeks of exposure to the mid-dose group; the severity of the diarrhea was dose-related. The gastrointestinal system was the primary target in animals dying early; numerous macroscopic and histological alterations were observed.

A benchmark dose (BMD) analysis of the incidence data for diarrhea was conducted; details of this analysis are presented in Appendix A. Using the female incidence data, a BMD (BMD_{10}) of 16.17 mg MMA/kg/day, which corresponds to a 10% increase in the incidence of diarrhea, was calculated; the 95% lower confidence limit on the BMD ($BMDL_{10}$) was 12.38 mg MMA/kg/day. The female incidence data were selected over the male data because the females may be more sensitive than the males. Thus, the intermediate-duration oral MRL of 0.1 mg MMA/kg/day is based on the $BMDL_{10}$ of 12.38 mg MMA/kg/day in female rats and an uncertainty factor of 100 (10 to account for animal to human extrapolation and 10 for human variability).

- An MRL of 0.01 mg MMA/kg/day has been derived for chronic-duration (365 days or longer) oral exposure to MMA.

The available data on the chronic toxicity of MMA in animals (no human data are available) suggest that the gastrointestinal tract and the kidney are the most sensitive targets. Diarrhea has been observed in rats and mice exposed to MMA in the diet for 2 years (Arnold et al. 2003). The NOAEL and LOAEL values for diarrhea are 3.0 and 25.7 mg MMA/kg/day in rats, respectively, and 24.9 and 67.1 mg MMA/kg/day

in mice, respectively. At 72.4 mg MMA/kg/day, necrotic, ulcerated, or perforated mucosa and metaplasia were observed in the cecum, colon, and rectum of rats. Squamous metaplasia was also observed in the cecum, colon, and rectum of mice exposed to 67.1 mg MMA/kg/day. Diarrhea was observed in dogs exposed via capsule to 2 mg MMA/kg/day for 52 weeks (Waner and Nyska 1988). The bolus administration used in the dog study probably increased the dog's sensitivity to MMA. In both the rats and mice, chronic administration of MMA resulted in an exacerbation of chronic progressive nephropathy. In female rats, significant increases in the severity of chronic progressive nephropathy were observed at 33.9 and 98.5 mg MMA/kg/day; the NOAEL was 3.9 mg MMA/kg/day (Arnold et al. 2003). In male mice, there was an increased incidence of slight progressive nephropathy at doses ≥6.0 mg MMA/kg/day; the NOAEL was 1.2 mg MMA/kg/day (Arnold et al. 2003; incidence data reported in Gur et al. 1991). Nephrocalcinosis was also observed in male mice exposed to ≥24.9 mg MMA/kg/day (Arnold et al. 2003). Other effects that have been observed following chronic exposure MMA include decreased weight gain in male and female rats exposed to 25.7/33.9 mg MMA/kg/day and higher (Arnold et al. 2003) and hypertrophy of the thyroid follicular epithelium in female rats exposed to ≥33.9 mg MMA/kg/day (Arnold et al. 2003). A variety of other lesions including peritonitis, pancreatitis, inflammation of the ureter, uterus, prostate, testes, epididymis, and seminal vesicles, hydronephrosis, pyelonephritis, and cortical tubular cystic dilation were also observed in rats; however, these alterations were probably secondary to the ulceration and perforation of the large intestine, which resulted in leaking of gastrointestinal contents into the abdominal cavity. Hyperplasia of the urinary bladder was also observed in rats exposed to 2.1 mg MMA/kg/day as MMA in drinking water for 2 years (Shen et al. 2003). Although hyperplasia of the urinary bladder is commonly observed in rats exposed to DMA, it was not observed in the Arnold et al. (2003) study at doses as high as 72.4 mg MMA/kg/day; thus, the significance of the results of the Shen et al. (2003) study is not known.

The lowest reliable LOAEL identified in the chronic oral MMA database was 6.0 mg MMA/kg/day for an increased incidence of progressive glomerulonephropathy in mice (Arnold et al. 2003). Although the investigators noted that the kidney lesions were consistent with the normal spectrum of spontaneous renal lesions and that there was no difference in character or severity of lesions between groups, ATSDR considers the dose-related increase in glomerulonephropathy to be treatment-related.

In the Arnold et al. (2003) study (incidence data reported in Gur et al. 1991), groups of 52 male and 52 female $B6C3F_1$ mice were exposed to 0, 10, 50, 200, or 400 ppm of MMA in the diet for 104 weeks. The average doses reported in Gur et al. (1991) were 0, 1.2, 6.0, 24.9, and 67.1 mg MMA/kg/day for males and 0, 1.4, 7.0, 31.2, and 101 mg MMA/kg/day for females. Body weights, food consumption, and

water intake were monitored regularly. Blood was taken at 3, 6, 12, 18, and 24 months for white cell counts. At sacrifice, complete necropsies were performed, including histological examination of at least 13 organs. No treatment-related increases in mortality were observed. Significant decreases in body weights were observed in males and females exposed to 67.1 or 101 mg MMA/kg/day, respectively; at week 104, the males and females weighed 17 and 23%, respectively, less than controls. Food consumption was increased in females exposed to 101 mg MMA/kg/day, and water consumption was increased in 67.1 mg MMA/kg/day males and 31.2 and 101 mg MMA/kg/day females. Loose and mucoid feces were noted in mice exposed to 67.1/101 mg MMA/kg/day. No changes were seen in white cell counts of either sex. Small decreases in the weights of heart, spleen, kidney, and liver weights were observed in some animals, but the decreases were not statistically significant. Squamous metaplasia of the cecum, colon, and rectum was observed at 67.1/101 mg MMA/kg/day. The incidence of metaplasia in the cecum, colon, and rectum were 29/49, 14/49, and 39/49 in males and 38/52, 17/52, and 42/52 in females; metaplasia was not observed in other groups of male or female mice. An increased incidence of progressive glomerulonephropathy (incidence of 25/52, 27/52, 38/52, 39/52, and 46/52 in the 0, 1.2, 6.0, 24.9, and 67.1 mg MMA/kg/day males, respectively) was observed in males; the incidence was significantly higher (Fisher Exact Test) than controls at ≥6.0 mg MMA/kg/day. Significant increases in the incidence of nephrocalcinosis was observed in the males at 24.9 and 67.1 mg MMA/kg/day (Fisher Exact Test) (incidences of 25/52, 30/52, 30/52, 45/52, and 45/51 in males and 0/52, 1/52, 1/52, 2/52, and 5/52 in females). A reduction in the incidence of cortical focal hyperplasia in the adrenal gland of male mice exposed to 67.1 mg MMA/kg/day was possibly related to MMA exposure; the toxicological significance of this effect is not known. Thus, this study identifies a NOAEL of 1.2 mg MMA/kg/day and a LOAEL of 6.0 mg MMA/kg/day for progressive glomerulonephropathy in male mice.

As described in greater detail in Appendix A, BMD was applied to the incidence data for progressive glomerulonephropathy in male mice using all available dichotomous models in EPA's Benchmark Dose Software (version 1.4.1) to calculate predicted doses associated with a 10% extra risk. As assessed by the Akaike's Information Criteria (AIC), the log-logistic model provided the best fit to the data. The predicted BMD_{10} and $BMDL_{10}$ are 2.09 and 1.09 mg MMA/kg/day. The $BMDL_{10}$ was selected as the point of departure and divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) to derive a chronic-duration oral MRL of 0.01 mg MMA/kg/day.

DMA. As discussed in greater detail in Section 2.2, urinary bladder effects characterized by cytotoxicity and regenerative proliferation and hyperplase have been observed in rats, but not in other species. The LOAELs for these effects are lower than the LOAELs for sensitive effects in other species. Additionally,

rats have a much greater capacity than other species to metabolize ingested DMA to form DMA(III) (a reactive intermediate) and TMAO (Cohen et al. 2006; Marafante et al. 1987b; Yoshida et al. 1998). It is likely that DMA(III) is the causative agent for the urothelial cytoxicity observed in rats (Cohen et al. 2006). Thus, rats were not considered a suitable model for humans and these data were not considered for derivation of MRLs for DMA.

There are limited data to assess the acute toxicity of DMA in species other than rats. Diarrhea, increased startle reflex, and ataxia were observed in mice exposed to a lethal gavage dose of 1,757 mg DMA/kg (Kaise et al. 1989); vomiting and diarrhea were also observed during the second week of a 52-week study in dogs exposed via capsule to 16 mg DMA/kg/day (Zomber et al. 1989). The remaining studies in the acute database are developmental toxicity studies in mice and rabbits. Rabbits appear to be more sensitive than mice to maternal and developmental effects. Gavage exposure to 48 mg DMA/kg/day on gestational days 7–19 resulted in maternal weight loss and abortion in approximately 75% of the does; no adverse effects were observed at 12 mg DMA/kg/day (Irvine et al. 2006). In mice, decreases in maternal body weight gain were observed at gavage doses of 200 mg DMA/kg/day on gestational days 7–16 (Rogers et al. 1981), decreases in fetal body weight, delays in ossification, and increased incidence of cleft palate were observed at 400 mg DMA/kg/day on gestational days 7–16 (Rogers et al. 1981) and fetal deaths, decreases in growth, and increased incidence of malformations were observed in mice administered 1,600 mg DMA/kg on gestational day 8 (Kavlock et al. 1985). The acute-duration database for DMA was not considered adequate for derivation of an oral MRL. The database is lacking a comprehensive toxicity study, which would be useful in establishing the critical target of toxicity. In a chronic-duration study in mice (Arnold et al. 2006), vacuolization was observed in the urinary bladder at ≥7.8 mg DMA/kg/day; it is not known if these effects would also be observed after acute-duration exposure. Thus, it is not known if systemic effects would occur at lower doses than the maternal developmental effects observed in rabbits exposed to 48 mg DMA/kg/day (Irvine et al. 2006); an acute-duration oral MRL for DMA is not recommended at this time.

Excluding rat studies, the database on the toxicity of DMA following intermediate-duration oral exposure is limited to a chronic study of dogs exposed to DMA via capsule 6 days/week for 52 weeks (Zomber et al. 1989). Diarrhea and vomiting were observed at 16 and 40 mg DMA/kg/day starting after the first week of exposure. A slight decrease in erythrocyte levels and increase in total leukocyte levels were observed in males exposed to 40 mg DMA/kg/day for 51 weeks. This dog study was not selected as the basis of an MRL because it is likely that bolus administration of DMA would increase sensitivity to the gastrointestinal effects.

- An MRL of 0.02 mg DMA/kg/day has been derived for chronic-duration (365 days or longer) oral exposure to DMA.

Two studies investigated the chronic-duration toxicity of DMA in a species other than rats. In dogs, diarrhea and vomiting were observed after 52 weeks of exposure to 16 or 40 mg As/kg/day (Zomber et al. 1989); no histological alterations were observed. In mice exposed to DMA in the diet for 2 years, vacuolization of the urothelium in the urinary bladder was observed at ≥7.8 mg DMA/kg/day and progressive glomerulonephropathy was observed at ≥37 mg DMA/kg/day (Arnold et al. 2006). As noted in Section 2.2, the vacuolization was not associated with cytotoxicity or proliferation. Because the bladder effects in mice occurred at the lowest adverse effect level for the database, it was selected as the critical effect and Arnold et al. (2006) was selected as the principal study.

In the Arnold et al. (2006) study, groups of 56 male and 56 female $B6C3F_1$ mice were exposed to 0, 8, 40, 200, or 500 ppm DMA in the diet for 2 years; the results of this study were also reported in an unpublished paper (Gur et al. 1989b) submitted to EPA under Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The investigators reported dietary doses of approximately 0, 1.3, 7.8, 37, and 94 mg DMA/kg/day. The following parameters were used to assess toxicity: clinical observations, body weight, food consumption, water consumption, differential leukocyte levels measured at 12, 18, and 24 months in mice in the control and 94 mg DMA/kg/day groups, organ weights (brain, kidneys, liver, and testes), and histopathological examination of major tissues and organs. No deaths or treatment-related clinical signs were observed. Decreases in body weight gain were observed in the male mice exposed to 94 mg DMA/kg/day; the difference was <10% and not considered adverse. An increase in water consumption was observed in males exposed to 94 mg DMA/kg/day during weeks 60–96. In the female mice exposed to 51 mg As/kg/day, a statistically significant decrease in lymphocytes and an increase in monocytes were observed at 24 months. Treatment related nonneoplastic alterations were observed in the urinary bladder and kidneys. In the urinary bladder, increases in the vacuolization of the superficial cells of the urothelium were observed in males exposed to 37 or 94 mg DMA/kg/day (0/44, 1/50, 0/50, 36/45, 48/48) and in females exposed to 7.8, 37, and 94 mg DMA/kg/day (1/45, 1/48, 26/43, 47/47, 43/43); incidence data reported in Gur et al. (1989b). An increased incidence of progressive glomerulonephropathy was observed in males at 37 mg DMA/kg/day (16/44, 22/50, 17/50, 34/45, 30/50) and an increased incidence of nephrocalcinosis was also observed in male mice at 94 mg DMA/kg/day (30/44, 25/50, 27/50, 29/50, 45/50). Neoplastic alterations were limited to an increased incidence of fibrosarcoma of the skin in females exposed to 94 mg DMA/kg/day (the incidence of 3/56, 0/55, 1/56,

1/56, and 6/56 in the 0, 1.3, 7.8, 37, and 94 mg DMA/kg/day groups, respectively); however, it was concluded that this lesion was not related to DMA exposure.

As described in detail in Appendix A, BMD analysis was applied to the incidence data for vacuolization of the urothelium in the urinary bladder of female mice using all available dichotomous models in EPA's Benchmark Dose Software (version 1.4.1) to calculate predicted doses associated with a 10% extra risk. As assessed by the AIC, the multi-stage model provided the best fit to the data. The predicted BMD_{10} and $BMDL_{10}$ are 2.68 and 1.80 mg DMA/kg/day. The $BMDL_{10}$ was selected as the point of departure and divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) to derive a chronic-duration oral MRL of 0.02 mg DMA/kg/day.

Roxarsone. A series of three National Toxicology Program (NTP) studies in rats and mice (NTP 1989b) and a study in dogs (Kerr et al. 1963) have examined the acute toxicity of roxarsone; adverse effects have also been reported within the first 2 weeks of a longer-term study in pigs (Rice et al. 1985; Kennedy et al. 1986). A single exposure study reported diarrhea and ataxia in rats and mice exposed to doses that exceeded the LD_{50} (NTP 1989b). In another study, no alterations in hematological parameters (only end point assessed) were found after 10 or 9 days of dietary exposure in rats and mice, respectively (NTP 1989b). In a 14-day study (NTP 1989b), a decrease in body weight gain and slight inactivity were observed in rats exposed to 32 mg roxarsone/kg/day and slight inactivity was observed in mice exposed to 42 mg roxarsone/kg/day; a decrease in body weight gain was also observed in mice exposed to 168 mg roxarsone/kg/day. The dog study was considered inadequate because a small number (n=3) of animals were tested and no control group was used. In a 30-day dietary exposure study in pigs (Rice et al. 1985; Kennedy et al. 1986), mild lethargy and ataxia were observed from day 7 forward and exercise-induced muscle tremors and clonic seizures were observed from day 11 forward in pigs exposed to 6.3 mg roxarsone/kg/day; equivocal evidence of myelin degeneration was also observed in pigs sacrificed after 11 days of exposure. These data clearly identify pigs as the most sensitive species following acute-duration oral exposure; in the absence of data to the contrary, it is assumed that pigs are a good model to predict the toxic potential of roxarsone in humans. Because the lowest dose tested in pigs was a serious LOAEL for neurotoxicity and a NOAEL for this effect was not identified, an acute-duration oral MRL cannot be derived for roxarsone.

As with the acute-duration database, pigs appear to be the most sensitive species; neurotoxicity has been observed at ≥6.3 mg roxarsone/kg/day. In a study reported by Rice et al. (1985) and Kennedy et al. (1986), exercise-induced muscle tremors and clonic convulsions were observed in pigs during the early

part of the study; when the pigs returned to a recumbent position, the seizures and tremors stopped. Paraparesis, evidenced by reluctance to rise and the pigs dragging their hindquarters on the ground, was observed at day 22; paraplegia was observed 2 days after exposure termination. In addition to these clinical signs of neuropathy, histological alterations consisting of myelin degeneration was observed in the spinal cord, peripheral nerves, and optic nerve. The lesions were first detected in the spinal cord on day 15 and in the peripheral nerves and optic nerve 2 days after exposure termination. The Rice et al. (1985) and Kennedy et al. (1986) studies did not identify a NOAEL. Muscle tremors were also observed in pigs exposed to 10 mg roxarsone/kg/day for 28 days (Edmonds and Baker 1986). This study was not designed to assess neurotoxicity and did not include histological examination of the spinal cord or nerves. Trembling, ataxia, and hyperexcitability were also observed in rats exposed to 64 mg roxarsone/kg/day for 13 weeks (NTP 1989b). Other effects that have been observed include tubular degeneration and focal regenerative hyperplasia in the kidney and decreased body weight in rats exposed to 32 mg roxarsone/kg/day for 13 weeks (NTP 1989b) and decreased body weight in mice at 136 mg roxarsone/kg/day for 13 weeks (NTP 1989b). The lowest identified adverse effect level is 6.3 mg roxarsone/kg/day for serious neurological effects in pigs (Kennedy et al. 1986; Rice et al. 1985) and is not suitable for the derivation of an intermediate-duration oral MRL.

The chronic toxicity of roxarsone has been examined in rats (NTP 1989b; Prier et al. 1963), mice (NTP 1989b; Prier et al. 1963), and dogs (Prier et al. 1963) in 2-year dietary exposure studies. None of these studies reported adverse effects at the highest doses tested; the highest NOAELs for each species are 10, 43, and 5 mg roxarsone/kg/day for rats, mice, and dogs, respectively. The results from shorter duration studies suggest that pigs are more sensitive to the neurotoxic effects of roxarsone than rats, mice, or dogs. Because no chronic duration pig studies were identified and deriving an MRL using a potentially less sensitive species may not be protective of human health, a chronic-duration oral MRL is not recommended at this time.

3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of arsenic. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not

the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of arsenic are indicated in Tables 3-1 and 3-3 and Figures 3-1 and 3-3. Because cancer effects could occur at lower exposure levels, Figures 3-1 and 3-3 also show a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

Chemical Forms of Concern. Analysis of the toxic effects of arsenic is complicated by the fact that arsenic can exist in several different oxidation states and many different inorganic and organic compounds. Most cases of human toxicity from arsenic have been associated with exposure to inorganic arsenic, so these compounds are the main focus of this profile.

The most common inorganic arsenical in air is arsenic trioxide (As_2O_3), while a variety of inorganic arsenates (AsO_4^{-3}) or arsenites (AsO_2^-) occur in water, soil, or food. A number of studies have noted differences in the relative toxicity of these compounds, with trivalent arsenites tending to be somewhat more toxic than pentavalent arsenates (Byron et al. 1967; Gaines 1960; Maitani et al. 1987a; Sardana et al. 1981; Willhite 1981). However, these distinctions have not been emphasized in this profile, for several reasons: (1) in most cases, the differences in the relative potency are reasonably small (about 2–3-fold), often within the bounds of uncertainty regarding NOAEL or LOAEL levels; (2) different forms of arsenic may be interconverted, both in the environment (see Section 6.3) and the body (see Section 3.4); and (3) in many cases of human exposure (especially those involving intake from water or soil, which are of greatest concern to residents near wastes sites), the precise chemical speciation is not known.

Gallium arsenide (GaAs) is another inorganic arsenic compound of potential human health concern, due to its widespread use in the microelectronics industry. Available toxicokinetic data suggest that although gallium arsenide is poorly soluble, it undergoes slow dissolution and oxidation to form gallium trioxide and arsenite (Webb et al. 1984, 1986). Therefore, the toxic effects of this compound are expected to be attributable to the arsenite that is liberated, plus the additional effects of the gallium species.

It is beyond the scope of this profile to provide detailed toxicity data on other less common inorganic arsenic compounds (e.g., As_2S_3), but these are expected to be of approximately equal or lesser toxicity than the oxycompounds, depending mainly on solubility (see Section 3.4).

Although organic arsenicals are usually viewed as being less toxic than the inorganics, several methyl and phenyl derivatives of arsenic that are widely used in agriculture are of possible human health concerns based on their toxicity in animal species (Arnold et al. 2003, 2006; NTP 1989b). Chief among these are monomethylarsonic acid (MMA) and its salts (monosodium methane arsonate [MSMA] and disodium methane arsonate [DSMA]), dimethylarsinic acid (DMA, also known as cacodylic acid) and its sodium salt (sodium dimethyl arsinite, or sodium cacodylate), and roxarsone (3-nitro-4-hydroxyphenylarsonic acid). However, it should be noted that food is the largest contributor to background intakes of organic arsenicals. Estimates on the concentration of organic arsenicals in the diet were not located; Cohen et al. (2006) estimated that the intake of DMA from food and water is <1 ng/kg/day. As with the inorganic compounds, there are toxicological differences between these various organic derivatives; because of these differences, the discussion of the health effects of MMA, DMA, and roxarsone are discussed separately. As discussed below, animals do not appear to be good quantitative models for inorganic arsenic toxicity in humans, but it is not known if this also applies to toxicity of organic arsenicals.

Several organic arsenicals are found to accumulate in fish and shellfish. These derivatives (mainly arsenobetaine and arsenocholine, also referred to as "fish arsenic") have been studied by several researchers and have been found to be essentially nontoxic (Brown et al. 1990; Cannon et al. 1983; Charbonneau et al. 1978; Kaise et al. 1985; Luten et al. 1982; Siewicki 1981; Tam et al. 1982; Yamauchi et al. 1986). Thus, these compounds are not considered further here.

Arsine (AsH_3) and its methyl derivatives, although highly toxic, are also not considered in this profile, since these compounds are either gases or volatile liquids that are unlikely to be present at levels of concern at hazardous waste sites.

Use of Animal Data. An additional complexity to the analysis of arsenic toxicity is that most laboratory animals appear to be substantially less susceptible to inorganic arsenic than humans. For example, chronic oral exposure of humans to inorganic arsenic at doses of 0.05–0.1 mg/kg/day is frequently associated with neurological (Barton et al. 1992; Goddard et al. 1992; Guha Mazumder et al. 1988; Haupert et al. 1996; Hindmarsh et al. 1977; Huang et al. 1985; Sass et al. 1993; Silver and Wainman 1952; Szuler et al. 1979; Tay and Seah 1975; Valentine et al. 1981) or hematological signs of arsenic toxicity (Glazener et al. 1968; Guha Mazumder et al. 1988; Prasad and Rossi 1995; Sass et al. 1993; Tay and Seah 1975), but no characteristic neurological or hematological signs of arsenism were detected in monkeys, dogs, or rats chronically exposed to arsenate or arsenite at doses of 0.7–2.8 mg As/kg/day (Byron et al. 1967; EPA 1980f; Heywood and Sortwell 1979). This may be because the studies were not conducted for a sufficient length of time, or because too few animals were used. Moreover, while there is good evidence that inorganic arsenic is carcinogenic in humans by both oral and inhalation routes, evidence of inorganic arsenic-induced carcinogenicity in animals is mostly negative, with the exception of studies in mice demonstrating transplacental carcinogenesis. For these reasons, quantitative dose-response data from animals are not judged to be reliable for determining levels of significant human exposure, and will be considered only briefly except when human data are lacking.

3.2.1 Inhalation Exposure

Most information on human inhalation exposure to arsenic derives from occupational settings such as smelters and chemical plants, where the predominant form of airborne arsenic is arsenic trioxide dust. One limitation to this type of study is that exposure data are usually difficult to obtain, especially from earlier time periods when exposure levels were higher than in recent years. This is further complicated by the fact that significant oral and dermal exposures are also likely to occur under these conditions and co exposure to other metals and chemicals is also common. Thus, studies of this type are, like virtually all epidemiological studies, subject to some limitations and uncertainties. Table 3-1 and Figure 3-1 summarize studies that provide the most reliable quantitative data on health effects in humans, along with several studies in animals exposed to arsenic trioxide and other inorganic arsenic compounds by the inhalation route. Data for DMA are shown in Table 3-2 and Figure 3-2. All exposure data are expressed as milligrams of arsenic (as the element) per cubic meter of air (mg As/m^3). These studies and others that provide useful qualitative information on health effects of inorganic and organic arsenicals are discussed below.

Table 3-1 Levels of Significant Exposure to Inorganic Arsenic - Inhalation

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)		LOAEL Serious (mg/m³)		Reference Chemical Form	Comments
ACUTE EXPOSURE										
Immuno/ Lymphoret										
1	Mouse (CD-1)	3 hr		0.123 F	0.271 F	(decreased pulmonary bactericidal activity and increased susceptibility to streptococcal infection)			Aranyi et al. 1985 As(+3)	
2	Mouse (CD-1)	5 d 3 hr/d		0.259 F	0.519 F	(decreased pulmonary bactericidal activity and increased susceptibility to streptococcal infection)			Aranyi et al. 1985 As(+3)	
Developmental										
3	Mouse (CFLP)	Gd 9-12 4 hr/d		0.2	2.2	(10% decreased average fetal body weight)	21.6	(increased fetal deaths, skeletal malformations, and retarded growth)	Nagymajtenyi et al. 1985 As(+3)	
INTERMEDIATE EXPOSURE										
Death										
4	Rat (CD)	14 pmd- Gd 19 7 d/wk 6 hr/d					20 F	(5/10 dams died)	Holson et al. 1999 As(+3)	
Systemic										
5	Rat (CD)	14 pmd- Gd 19 7 d/wk 6 hr/d	Resp	2 F	8 F	(rales, dried red material around nose)			Holson et al. 1999 As(+3)	
			Bd Wt	2 F	8 F	(decreased body weight gain during gestation)				

Table 3-1 Levels of Significant Exposure to Inorganic Arsenic - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
6	Rat (CD)	14 pmd- Gd 19 7 d/wk 6 hr/d	Resp	0.9 F	8 F (rales)	20 F (labored breathing, gasping)	Holson et al. 1999 As(+3)	
			Gastro	8 F		20 F (gross gastrointestinal lesions)		
			Bd Wt	8 F		20 F (drastic decrease body weight)		
Immuno/ Lymphoret								
7	Mouse (CD-1)	4 wk 5 d/wk 3 hr/d		0.126 F	0.245 F (decreased pulmonary bactericidal activity)		Aranyi et al. 1985 As(+3)	
Reproductive								
8	Rat (CD)	14 pmd- Gd 19 7 d/wk 6 hr/d		8 F			Holson et al. 1999 As(+3)	
9	Rat (CD)	14 pmd- Gd 19 7 d/wk 6 hr/d		20 F			Holson et al. 1999 As(+3)	
Developmental								
10	Rat (CD)	14 pmd- Gd 19 7 d/wk 6 hr/d		8			Holson et al. 1999 As(+3)	

Table 3-1 Levels of Significant Exposure to Inorganic Arsenic - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)		LOAEL Serious (mg/m³)		Reference Chemical Form	Comments
11	Rat (CD)	14 pmd- Gd 19 7 d/wk 6 hr/d		8			20	(marked increase in post-implantation loss and marked decrease in viable fetuses)	Holson et al. 1999 As(+3)	
CHRONIC EXPOSURE										
Systemic										
12	Human	23 yr (avg) (occup)	Cardio				0.36 M	(increased incidence of vasospasticity and clinical Raynaud's phenomenon)	Lagerkvist et al. 1986 As(+3)	
13	Human	0.5-50 yr (occup)	Resp	0.613					Perry et al. 1948 As(+3)	
			Dermal		0.078	(mild pigmentation keratosis of skin)	0.613	(gross pigmentation with hyperkeratinization of exposed areas, wart formation)		
Neurological										
14	Human	28 yr (avg) (occup)			0.31 M	(decreased nerve conduction velocity)			Lagerkvist and Zetterlund 1994 As(+3)	
Cancer										
15	Human	1- >30 yr (occup)					0.213 M	(CEL: lung cancer)	Enterline et al. 1987a As(+3)	
16	Human	19.5 yr (avg) (occup)					0.069 M	(CEL: lung cancer)	Enterline et al. 1987b As(+3)	

Table 3-1 Levels of Significant Exposure to Inorganic Arsenic - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
17	Human	3 mo- >30 yr (occup)				0.2 M (CEL: lung cancer)	Jarup and Pershagen 1991 As(+3)	
18	Human	3 mo- >30 yr (occup)				0.05 M (CEL: lung cancer)	Jarup et al. 1989 As(+3)	
19	Human	1- >30 yr (occup)				0.38 M (CEL: lung cancer)	Lee-Feldstein 1986 As(+3)	
20	Human	>25 yr (occup)				0.29 M (CEL: lung cancer)	Lubin et al. 2000 As(+3)	
21	Human	14.8 yr (avg) (occup)				0.3 M (CEL: lung cancer)	Welch et al. 1982 As(+3)	

a The number corresponds to entries in Figure 3-1.

avg = average; Bd Wt = body weight; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); F = female; Gastro = gastrointestinal; Gd = gestation day; hr = hour(s); Immuno/Lymphoret = immunological/lymphoreticular; LOAEL = lowest-observable-adverse-effect level; M = male; mo = month(s); NOAEL = no-observable-adverse-effect level; NS = not specified; occup = occupational; pmd = pre-mating day; Resp = respiratory; wk = week(s); yr = year(s)

Figure 3-1 Levels of Significant Exposure to Inorganic Arsenic - Inhalation

Acute (≤14 days)

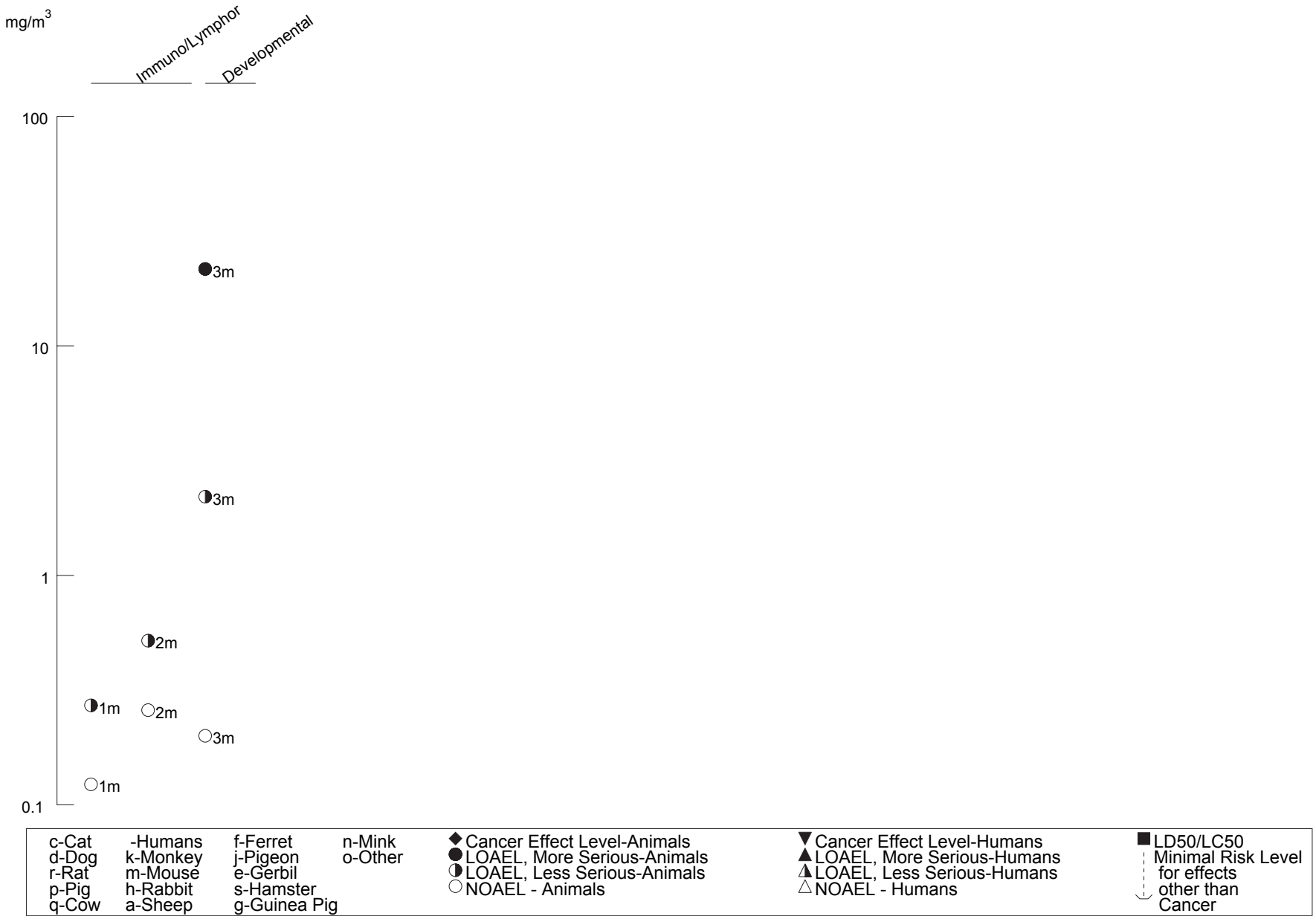


Figure 3-1 Levels of Significant Exposure to Inorganic Arsenic - Inhalation *(Continued)*

Intermediate (15-364 days)

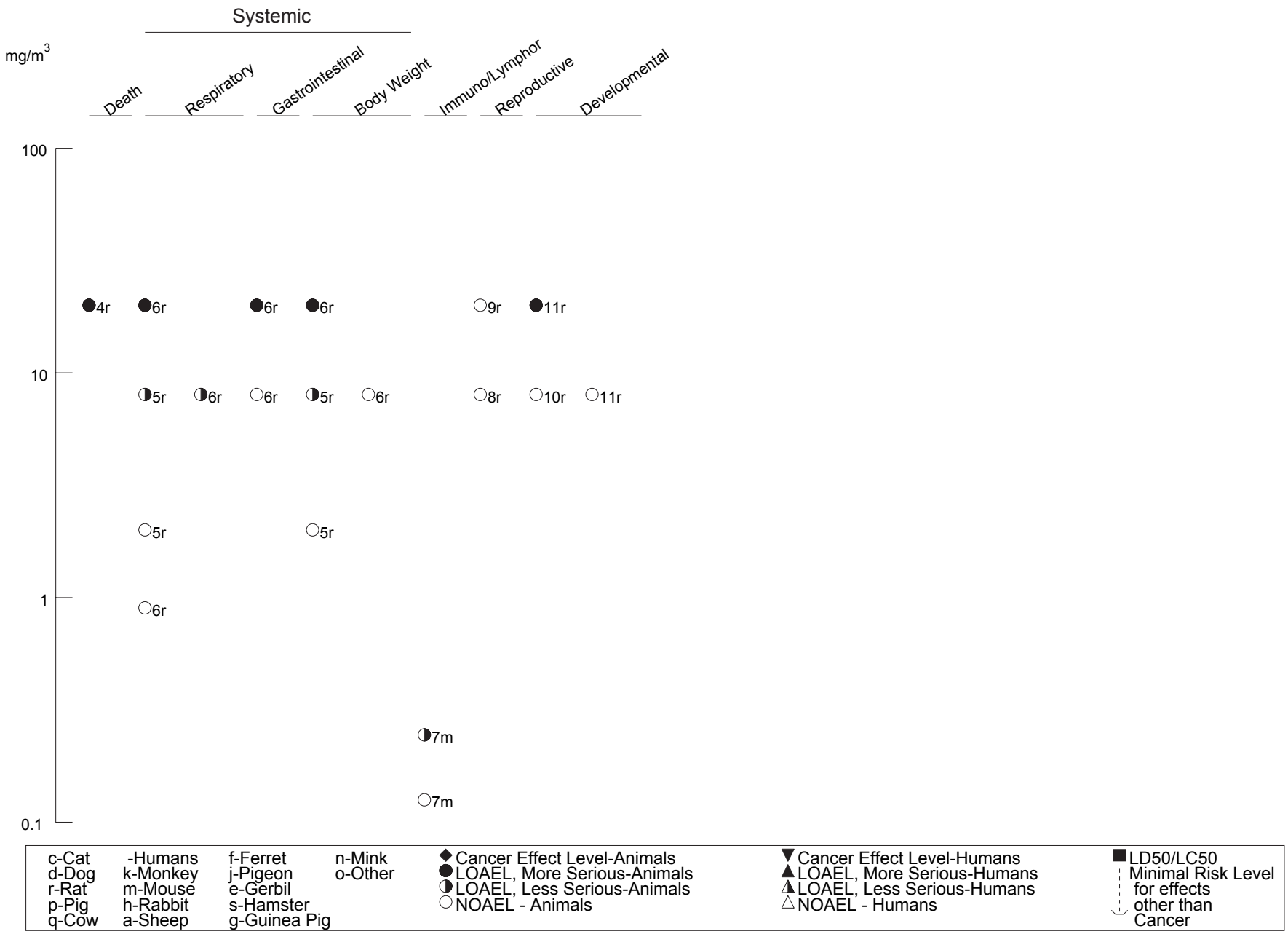
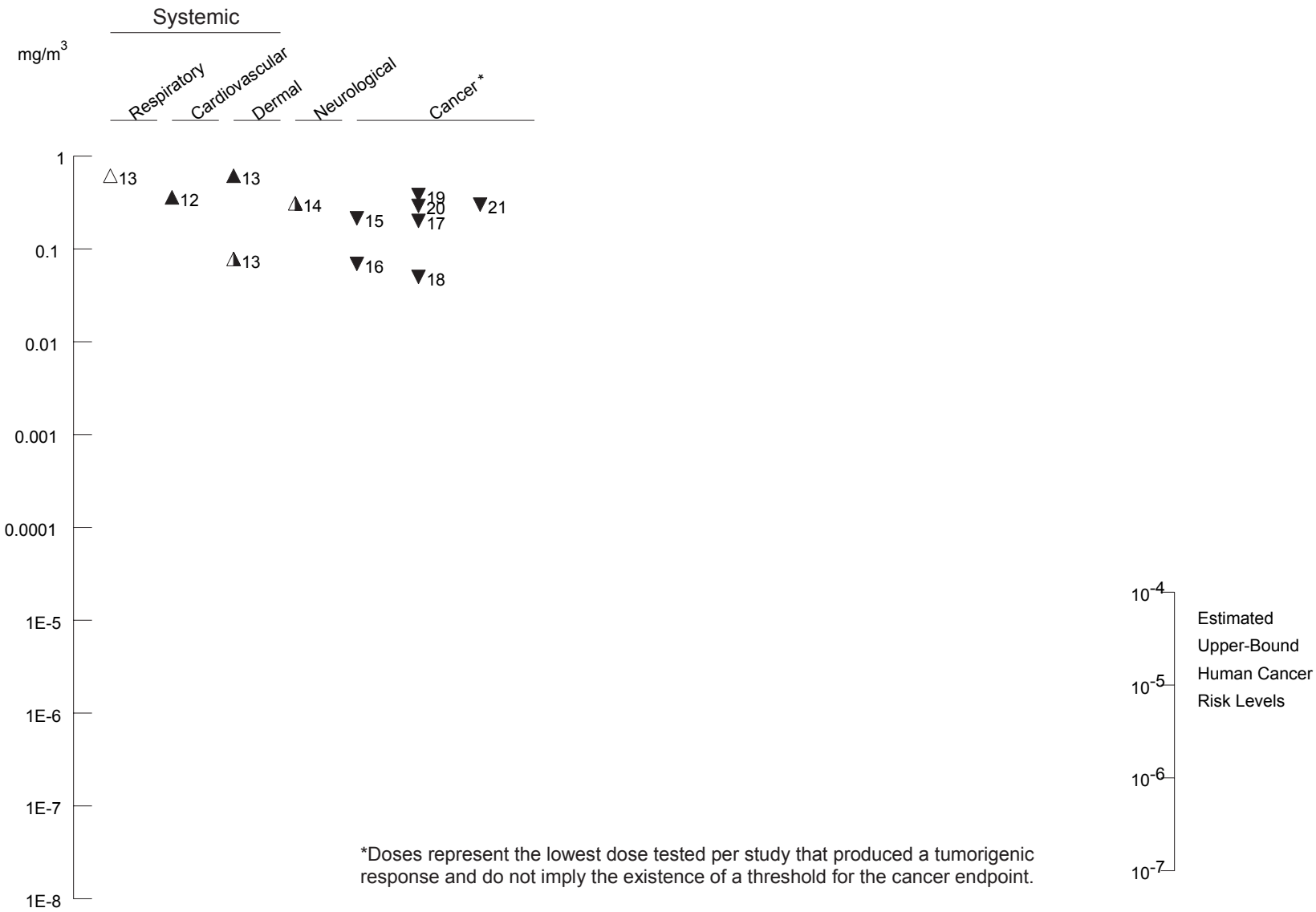


Figure 3-1 Levels of Significant Exposure to Inorganic Arsenic - Inhalation *(Continued)*

Chronic (≥365 days)



*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint.

c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level for effects other than Cancer
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◮ LOAEL, Less Serious-Humans	
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	
q-Cow	a-Sheep	g-Guinea Pig				

Table 3-2 Levels of Significant Exposure to Dimethylarsinic Acid - Inhalation

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Rat (Sherman)	2 hr				3900 F (LC50)	Stevens et al. 1979 DMA	
Systemic								
2	Rat (Sherman)	2 hr	Resp			4000 (respiratory distress)	Stevens et al. 1979 DMA	
			Gastro		4000 (diarrhea)			
			Dermal	4100	6900 F (erythematous lesions of ears and feet)			
			Ocular		4000 (eye encrustation)			
			Bd Wt		4000 (unspecified decrease in body weight)			
3	Mouse (Swiss-Webster)	5 min	Resp		3150 M (RD50)		Stevens et al. 1979 DMA	
INTERMEDIATE EXPOSURE								
Systemic								
4	Rat (Sprague-Dawley)	6 hr/d 5 d/wk 67-68 exposures	Resp	10	34 (intracytoplasmic eosinophilic globules in nasal turbinates)		Whitman 1994 DMA	
			Cardio	100				
			Gastro	100				
			Hemato	100				
			Hepatic	100				
			Renal	100				
			Endocr	100				
			Dermal	100				

Table 3-2 Levels of Significant Exposure to Dimethylarsinic Acid - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
			Bd Wt	100				

a The number corresponds to entries in Figure 3-2.

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); DMA = dimethylarsinic acid; F = female; Gastro = gastrointestinal; Hemato = hematological; hr = hour(s); LC50 = lethal concentration, 50% kill; LOAEL = lowest-observable-adverse-effect level; M = male; min = minute(s); NOAEL = no-observable-adverse-effect level; RD50 = 50% decrease in respiration rate; Resp = respiratory; wk = week(s); yr = year(s)

Figure 3-2 Levels of Significant Exposure to Dimethylarsinic Acid - Inhalation

Acute (≤14 days)

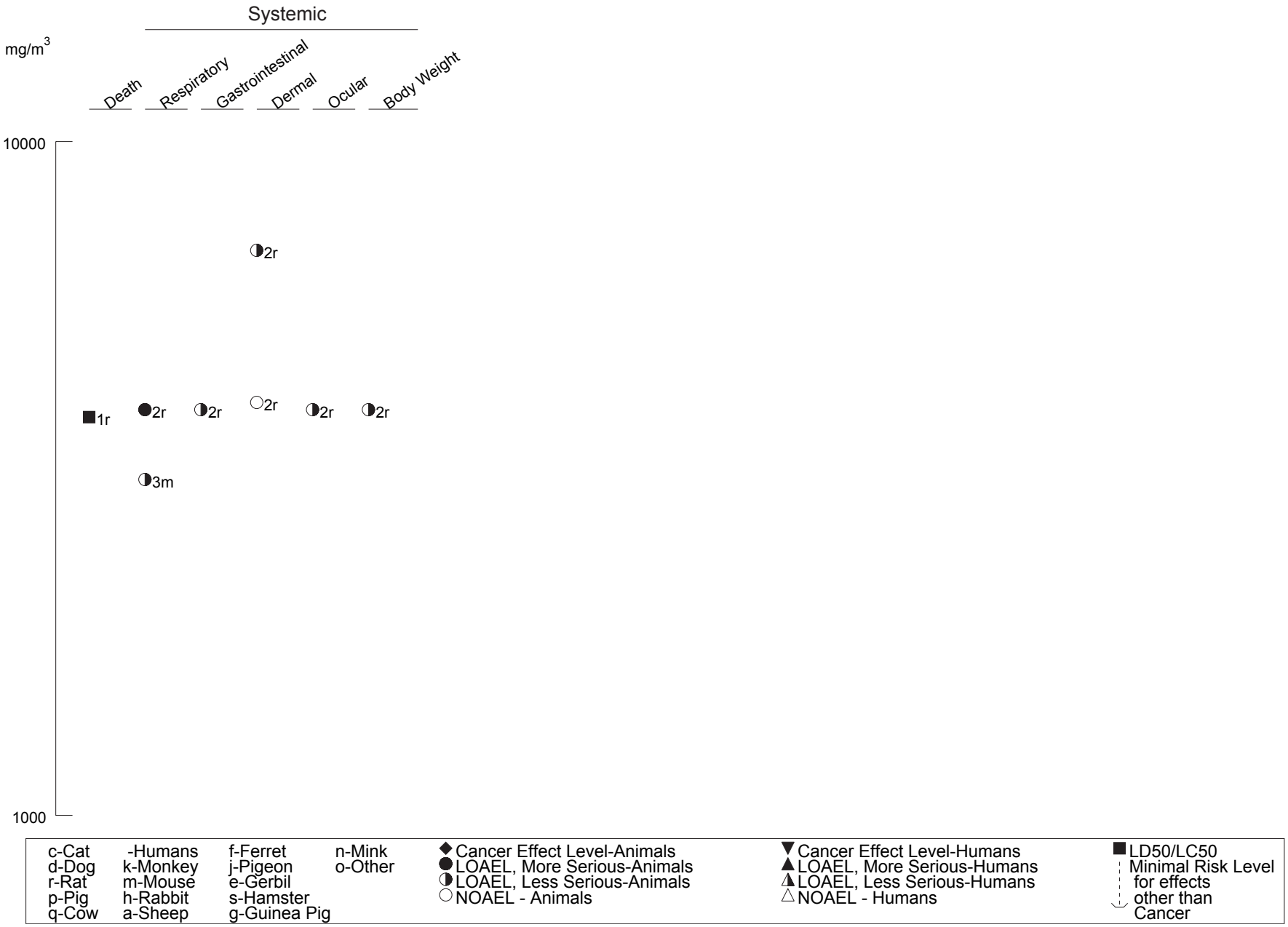
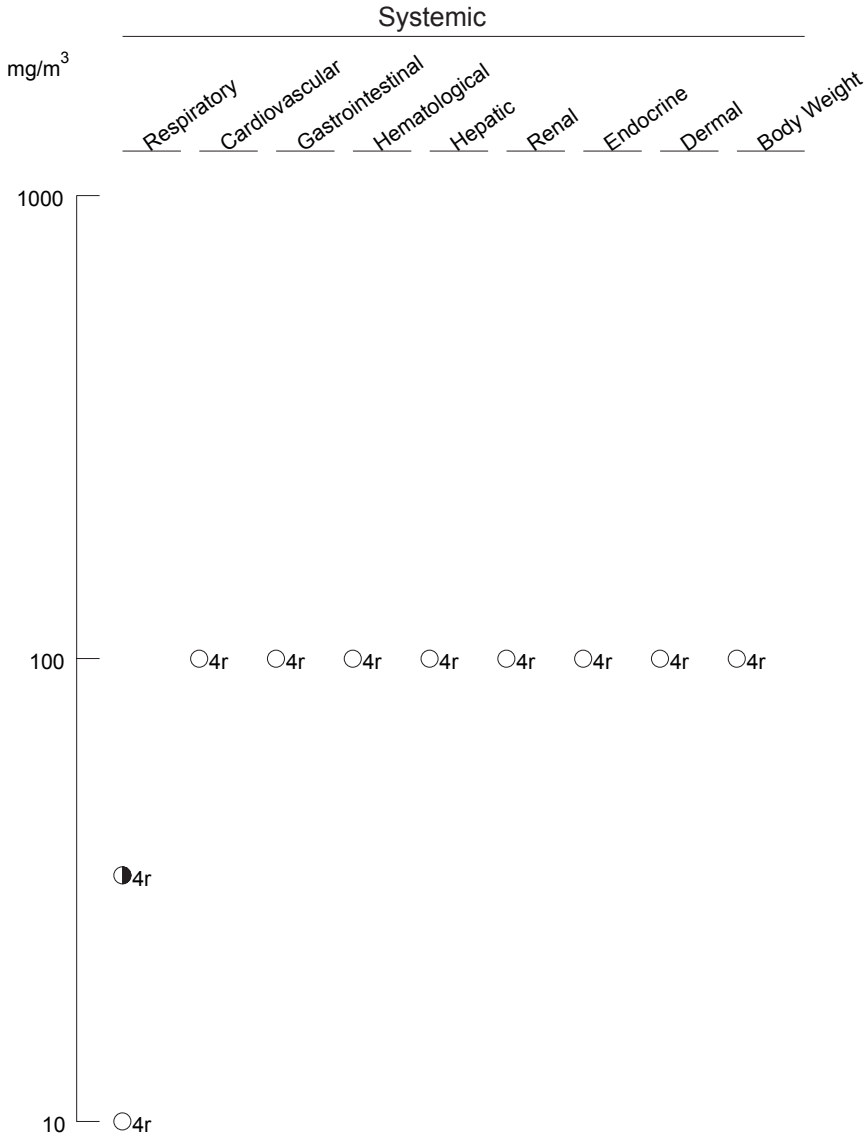


Figure 3-2 Levels of Significant Exposure to Dimethylarsinic Acid - Inhalation *(Continued)*

Intermediate (15-364 days)



c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level for effects other than Cancer
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◭ LOAEL, Less Serious-Humans	
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	
q-Cow	a-Sheep	g-Guinea Pig				

3.2.1.1 Death

Inorganic Arsenicals. Although there are many studies of humans exposed to arsenic in air, no cases of lethality from short-term exposure were located. This suggests that death is not likely to be of concern following acute exposure, even at the very high exposure levels (1–100 mg As/m^3) found previously in the workplace (e.g., Enterline and Marsh 1982; Järup et al. 1989; Lee-Feldstein 1986). Delayed lethality from chronic exposure attributable to increased risk of cardiovascular disease or lung cancer is discussed below in Sections 3.2.1.2 and 3.2.1.7, respectively. The only report of a lethal effect of inhaled inorganic arsenic in animals was a developmental toxicology study in which four of nine pregnant rats died, and one rat was euthanized in extremis, between days 12 and 19 of gestation after 30–35 days of exposure to an aerosol of arsenic trioxide at an exposure concentration of 20 mg As/m^3 (Holson et al. 1999). These animals exhibited severe hyperemia and plasma discharge into the intestinal lumen at autopsy. In this same study, there was 100% mortality in groups of 10 pregnant rats after 1 day of exposure to concentrations ≥100 mg/m^3 (76 mg As/m^3).

Organic Arsenicals. No studies were located regarding death in humans after inhalation exposure to organic arsenicals. A 2-hour LC_{50} of 3,900 mg DMA/m^3 was calculated for DMA in female rats (Stevens et al. 1979). This LC_{50} is shown in Table 3-2 and Figure 3-2. Male rats and mice of both sexes were less susceptible, with only a few deaths after 2-hour exposures as high as 6,900 mg DMA/m^3 in rats and 6,400 mg DMA/m^3 in mice (Stevens et al. 1979). The cause of death was not specified, but was probably due to lung injury (see Section 3.2.1.2). No deaths were observed among rats and mice exposed to DSMA (the disodium salt of MMA) at concentrations up to 6,100 mg DSMA/m^3 in rats and 6,900 mg DSMA/m^3 in mice (Stevens et al. 1979). Chamber atmospheres at these high concentrations were so dense that it was difficult to see the animals clearly. These data indicate that there is no significant risk of acute lethality from concentrations of DMA or MMA that might be encountered in the environment or the workplace.

3.2.1.2 Systemic Effects

The highest NOAEL values and all reliable LOAEL values for systemic effects from inhalation exposure to inorganic arsenicals in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1, while the corresponding data for DMA are shown in Table 3-2 and Figure 3-2.

Respiratory Effects.

Inorganic Arsenicals. Workers exposed to arsenic dusts in air often experience irritation to the mucous membranes of the nose and throat. This may lead to laryngitis, bronchitis, or rhinitis (Dunlap 1921; Morton and Caron 1989; Pinto and McGill 1953), and very high exposures (characteristic of workplace exposures in the past) can cause perforation of the nasal septum (Dunlap 1921; Pinto and McGill 1953; Sandstrom et al. 1989). Despite the known respiratory irritant effects of arsenic, there have been few systematic investigations of respiratory effects in humans exposed to arsenic. Perry et al. (1948) found no difference in chest x-rays or respiratory performance (vital capacity and exercise-tolerance tests) between unexposed and exposed workers in a cross-sectional study at a factory where sodium arsenite was prepared. The NOAEL of 0.613 mg As/m^3 for respiratory effects in this study is shown in Table 3-1 and plotted in Figure 3-1.

Increased mortality due to respiratory disease has been reported in some cohort mortality studies of arsenic-exposed workers, but no conclusive evidence of an association with arsenic has been produced. In studies of workers exposed to arsenic trioxide at the Anaconda copper smelter in Montana, mortality due to noncancer respiratory disease (e.g., emphysema) was significantly increased compared to the general population (Lee-Feldstein 1983; Lubin et al. 2000; Welch et al. 1982). However, the data were not adjusted for smoking (a well-known confounder for respiratory disease), and analysis of the data with respect to arsenic exposure level did not show a clear dose-response. Similarly, Enterline et al. (1995) found a significant excess of nonmalignant respiratory disease mortality in workers at the ASARCO copper smelter in Tacoma, Washington, but only a slight negative relation to cumulative arsenic exposure. Xuan et al. (1993) found an increase in the relative risk of mortality from pneumoconiosis associated with arsenic exposure in a cohort of tin miners in China. However, this finding was based on a small number of observations (n=32), a clear exposure-response relationship with arsenic was not established, and the miners experienced confounding exposures to dust (a known risk factor for pneumoconiosis) and to radon. These studies were all considered to be inconclusive as to the relationship between inhaled inorganic arsenic and respiratory disease.

Respiratory symptoms were observed in a study of developmental effects in rats. Pregnant female rats exposed to arsenic trioxide dust starting 14 days prior to mating and continuing through mating and gestation exhibited rales at 8 mg As/m^3 and labored breathing and gasping at 20 mg As/m^3, with no symptoms at 2 mg As/m^3 (Holson et al. 1999). The lungs were examined by gross necropsy and no lesions were found. Intratracheal instillation of arsenic trioxide (13 mg As/kg) or gallium arsenide (1.5–

52 mg As/kg) can cause marked irritation and hyperplasia in the lungs of rats and hamsters (Goering et al. 1988; Ohyama et al. 1988; Webb et al. 1986, 1987). Since this sort of response is produced by a number of respirable particulate materials, it is likely that the inflammatory response is not specifically due to the arsenic.

Organic Arsenicals. No studies were located regarding respiratory effects in humans exposed to organic arsenicals. Short-term exposure of rats and mice to high concentrations (≥4,000 mg/m^3) of DMA caused respiratory distress, and necropsy of animals that died revealed bright red lungs with dark spots (Stevens et al. 1979). Respiratory distress was also observed in rats and mice exposed to high levels (≥6,100 mg/m^3) of the disodium salt of MMA (Stevens et al. 1979), although none of the MMA-exposed animals died. Respiratory distress appears to be associated with inhalation of very high concentrations of organic arsenicals. In 5-minute whole-body plethysmography trials, DMA and the disodium salt of MMA had RD_{50} (concentration calculated to produce a 50% decrease in respiration rate) values of 3,150 and 1,540 mg/m^3, respectively (Stevens et al. 1979). Based on these RD_{50} values, neither DMA nor MMA is considered to be a potent respiratory irritant. At low concentrations of DMA (34 or 100 mg DMA/m^3), an increase in intracytoplasmic eosinophilic globules were found in the nasal turbinates of rats exposed to DMA 6 hours/day, 5 days/week for 67–68 exposures (Whitman 1994).

Cardiovascular Effects.

Inorganic Arsenicals. There is some evidence from epidemiological studies that inhaled inorganic arsenic can produce effects on the cardiovascular system. Cardiovascular effects following oral exposure to arsenic are well known (see Section 3.2.2.2). A cross-sectional study of workers exposed to an estimated time-weighted average of 0.36 mg As/m^3 (as arsenic trioxide) at the Ronnskar copper smelter in Sweden for an average of 23 years showed that smelter workers had significantly increased incidences of Raynaud's phenomenon (a peripheral vascular disease characterized by spasm of the digital arteries and numbness of the fingers) and showed increased vasospasticity (constriction of blood vessels) in response to cold when tested in the fingers (Lagerkvist et al. 1986). A follow-up study conducted 2–3 years later found that vasospasticity measurements in exposed workers had improved concurrent with a reduction in arsenic exposure levels, although symptoms of peripheral vascular effects (cold hands or feet, white fingers, numbness in fingers or feet) were still common (Lagerkvist et al. 1988). A cross-sectional study including 46 workers in Denmark with varying, unquantified occupational exposure to arsenic in different occupations found that systolic blood pressure was significantly increased in the arsenic workers (median=125 mmHg) compared with controls (median=117 mmHg) (Jensen and Hansen 1998). Diastolic

pressure was also increased in this study (77.9 vs. 74.7 mmHg), although the difference from controls was not statistically significant.

Cohort mortality studies of arsenic-exposed workers at the ASARCO copper smelter in Tacoma, Washington (Enterline et al. 1995), Anaconda copper smelter in Montana (Lee-Feldstein 1983; Welch et al. 1982), Ronnskar copper smelter in Sweden (Wall 1980), orchard workers in Washington state (Tollestrup et al. 1995), and tin miners in China (Qiao et al. 1997; Xuan et al. 1993) have all reported increased risk of mortality from cardiovascular disease, specifically ischemic heart disease and cerebrovascular disease, in the cohorts studied. However, none of these studies provided conclusive evidence that the observed increase in risk was due to arsenic exposure. The studies in the ASARCO and Anaconda copper smelter workers failed to find a clear dose-response relationship with arsenic (Enterline et al. 1995; Welch et al. 1982), while a follow-up study of the Ronnskar smelter workers not only found lack of a dose-response, but also that the risk of cardiovascular disease was no longer elevated in the cohort (Järup et al. 1989). The studies in orchard workers and tin miners were limited by confounding exposures to copper, lead, and radon, respectively (Qiao et al. 1997; Tollestrup et al. 1995). The risk of cardiovascular disease mortality in the tin miners not only showed no dose-response relationship with arsenic exposure, but was positively associated with radon exposure, suggesting that radon may have been responsible for the increased cardiovascular risk in this cohort (Xuan et al. 1993).

The LOAEL for Raynaud's phenomenon and vasospasticity identified by Lagerkvist et al. (1986) is shown in Table 3-1 and Figure 3-1. No studies were located regarding cardiovascular effects in animals after inhalation exposure to inorganic arsenic.

Organic Arsenicals. No studies were located regarding cardiovascular effects in humans after inhalation exposure to organic arsenicals. No histological alterations were observed in the hearts of rats exposed to 100 mg DMA/m^3 for 67–68 exposures (Whitman 1994).

Gastrointestinal Effects.

Inorganic Arsenicals. Several case studies have reported nausea, vomiting, and diarrhea in workers with acute arsenic poisoning following occupational inhalation exposure (Beckett et al. 1986; Bolla-Wilson and Bleecker 1987; Ide and Bullough 1988; Morton and Caron 1989; Pinto and McGill 1953). Although gastrointestinal effects are not typically associated with arsenic poisoning by inhalation (Pinto and McGill 1953), such effects are a common feature of oral ingestion of high doses of arsenic (see Section 3.2.2.2),

and it is possible that mucociliary transport of arsenic dust from the lungs to the gut could be responsible for the effects in these cases. Exposure levels were not reliably estimated for any of these cases.

The only report of gastrointestinal effects of inhaled inorganic arsenic in animals was a developmental toxicology study in which four of nine pregnant rats died, and one rat was euthanized *in extremis*, between days 12 and 19 of gestation after 30–35 days of exposure to an aerosol of arsenic trioxide at an exposure concentration of 20 mg As/m^3 (Holson et al. 1999). These animals exhibited severe hyperemia and plasma discharge into the intestinal lumen at autopsy. Exposure to 8 mg As/m^3 did not produce gross gastrointestinal lesions.

Organic Arsenicals. Data regarding gastrointestinal effects in people exposed to organic arsenic in the air are limited. The frequency of gastrointestinal complaints was no higher than controls in workers exposed to arsanilic acid (i.e., 4-aminophenyl arsonic acid) at mean concentrations up to 0.17 mg/m^3 in a chemical factory (Watrous and McCaughey 1945). However, this sort of data might easily be biased by workers who chose not to complain about minor symptoms, so no conclusion can be reached. Rats and mice exposed to very high levels (above 3,000 mg/m^3) of MMA (disodium salt) or DMA experienced diarrhea (Stevens et al. 1979). The diarrhea could be due to transport of inhaled particulate material from the lungs to the gastrointestinal system or to direct ingestion of the compound (e.g., from grooming of the fur). No gastrointestinal effects were observed in rats repeatedly exposed to 100 mg DMA/m^3 6 hours/day, 5 days/week for 67–68 exposures (Whitman 1994).

Hematological Effects.

Inorganic Arsenicals. Although anemia is a common feature of arsenic poisoning following oral exposure in humans (see Section 3.2.2.2), case studies of workers with arsenic poisoning from occupational inhalation exposure reported no effects on red blood cell count (Beckett et al. 1986; Bolla-Wilson and Bleecker 1987; Ide and Bullough 1988; Morton and Caron 1989). The reason for this apparent route specificity is not clear, but might simply be related to dose. No studies were located regarding hematological effects in animals after inhalation exposure to inorganic arsenicals.

Organic Arsenicals. No effect on levels of hemoglobin, red cells, or white cells was detected in the blood of manufacturing workers (323 counts in 35 workers) exposed to airborne arsanilic acid dusts at a mean concentration of 0.17 mg/m^3 in the workplace (Watrous and McCaughey 1945). Controls were an unspecified number of unexposed manufacturing workers with 221 complete blood counts. No

hematological alterations were observed in rats exposed to 100 mg DMA/m^3 for an intermediate duration (Whitman 1994).

Musculoskeletal Effects.

Inorganic Arsenicals. Few data were located regarding musculoskeletal effects associated with inhalation exposure to inorganic arsenic, and none to suggest the existence of any such effects. Electromyographic examination of the calves and feet showed no differences between control and arsenic-exposed workers in a cross-sectional study of workers at the Ronnskar copper smelter in Sweden (Blom et al. 1985). No studies were located regarding musculoskeletal effects in animals after inhalation exposure to inorganic arsenicals.

Organic Arsenicals. No studies were located regarding musculoskeletal effects in humans or animals after inhalation exposure to organic arsenicals.

Hepatic Effects.

Inorganic Arsenicals. There is no evidence that inhaled inorganic arsenic produces effects on the liver, although few data are available. Case studies of workers with inhalation arsenic poisoning that included liver function tests did not find any evidence of hepatic dysfunction (Bolla-Wilson and Bleecker 1987; Ide and Bullough 1988). No studies were located regarding hepatic effects in animals after inhalation exposure to inorganic arsenicals.

Organic Arsenicals. No studies were located regarding hepatic effects in humans after inhalation exposure to organic arsenicals. No histological alterations were observed in the livers of rats exposed to 100 mg DMA/m^3 for 67–68 exposures (Whitman 1994).

Renal Effects.

Inorganic Arsenicals. The limited data available do not suggest any relationship between inhalation of inorganic arsenic and kidney effects. A cross-sectional study of renal function parameters in glass factory workers exposed to arsenic (concentrations unknown) found no meaningful differences from controls in urinary levels of several proteins (albumin, retinol binding protein, β_2-microglobulin, brush-border antigen) used as markers of glomerular damage or tubular cell exfoliation (Foà et al. 1987). Routine

clinical urinalysis was normal when included in case studies of workers with inhalation arsenic poisoning (Ide and Bullough 1988; Morton and Caron 1989). No studies were located regarding renal effects in animals after inhalation exposure to inorganic arsenicals.

Organic Arsenicals. No studies were located regarding renal effects in humans after inhalation exposure to organic arsenicals. No renal effects were reported in rats exposed to 100 mg DMA/m^3 6 hours/day, 5 days/week for 67–68 exposures (Whitman 1994).

Dermal Effects.

Inorganic Arsenicals. Dermatitis has frequently been observed in industrial workers exposed to inorganic arsenic in the air, with the highest rates occurring in the workers with the greatest arsenic exposure (Cöl et al. 1999; Dunlap 1921; Holmqvist 1951; Lagerkvist et al. 1986; Pinto and McGill 1953). Limited quantitative information is available regarding the exposure levels that produce dermatitis, and the high likelihood of co-exposure by the dermal route makes dose-response analysis difficult. A cross-sectional study of workers at a factory where sodium arsenite was prepared found that workers with the highest arsenic exposure (mean air levels ranging from 0.384 to 1.034 mg As/m^3 and estimated to average 0.613 mg As/m^3) tended to be grossly pigmented with hyperkeratinization of exposed skin and to have multiple warts (Perry et al. 1948). In the same study, workers with lower arsenic exposure (estimated to average 0.078 mg As/m^3) were much less affected, but still had a higher incidence of pigmentation keratosis than controls. LOAEL values identified by Perry et al. (1948) and Mohamed (1998) are shown in Table 3-1 and Figure 3-1. NOAEL values for dermal irritation have not been identified. Dermal effects (hyperkeratoses, hyperpigmentation) are also very common in people exposed to inorganic arsenic by the oral route (see Section 3.2.2.2). No studies were located on dermal effects in animals after inhalation exposure to inorganic arsenicals.

Organic Arsenicals. Data regarding dermal effects in people exposed to organic arsenic in the air are limited. Complaints of keratosis were roughly 2-fold higher than unexposed controls in female packaging workers exposed to arsanilic acid at an average concentration of 0.065 mg/m^3 and in male manufacturing workers exposed to an average concentration of 0.17 mg/m^3 in a chemical factory (Watrous and McCaughey 1945). Limitations in study methodology (e.g., alternate sources of effects were not investigated, workers might choose not to report minor complaints to company officials) make the reliability of this observation uncertain. Female rats exposed to DMA at 6,900 mg/m^3 developed erythematous lesions on the feet and ears (Stevens et al. 1979); these lesions did not develop in females

exposed at lower concentrations (4,100 mg/m^3) or males. It seems likely that these effects were due to direct irritation from dermal contact with the dust. No dermal effects were observed in rats repeatedly exposed to lower levels of DMA (100 mg/m^3) (Whitman 1994).

Ocular Effects.

Inorganic Arsenicals. Chemical conjunctivitis, characterized by redness, swelling, and pain, has been observed in workers exposed to arsenic dusts in air, usually accompanied by facial dermatitis (Dunlap 1921; Pinto and McGill 1953). No information was located regarding air levels of arsenic that produce this effect. No studies were located on ocular effects in animals after inhalation exposure to inorganic arsenicals.

Organic Arsenicals. No studies were located on ocular effects in humans after inhalation exposure to organic arsenicals. Rats and mice exposed to high concentrations of DMA (≥4,000 mg/m^3) developed an encrustation around the eyes (Stevens et al. 1979). It seems likely that these effects were due to direct irritation from ocular contact with the dust.

Body Weight Effects.

Inorganic Arsenicals. No studies were located on body weight effects in humans after inhalation exposure to inorganic arsenicals. Female rats exposed to arsenic trioxide dust starting 14 days before mating and continuing through mating and gestation showed a marked decrease in body weight and food consumption at 20 mg As/m^3 (preliminary study) and a smaller decrease at 8 mg As/m^3 (definitive study), with no effect at 2 mg As/m^3 (Holson et al. 1999).

Organic Arsenicals. No studies were located on body weight effects in humans after inhalation exposure to organic arsenicals. Rats and mice exposed to high concentrations of DMA (≥4,000 mg/m^3) for 2 hours had an unspecified decrease in body weight gain during the subsequent 14 days (Stevens et al. 1979). No alterations in body weight gain were observed in rats exposed to 100 mg DMA/m^3 for 67–68 exposures (Whitman 1994).

3.2.1.3 Immunological and Lymphoreticular Effects

Inorganic Arsenicals. A single study was located regarding the immunological and lymphoreticular effects of inhaled inorganic arsenic in humans. Bencko et al. (1988) detected no abnormalities in serum levels of immunoglobins in 47 workers exposed to arsenic (exposure levels not measured) in a coal-burning power plant. However, serum levels of other proteins such as transferrin, orosomucoid, and ceruloplasmin were significantly elevated compared to levels in a group of 27 workers from a different plant in which the arsenic content in the coal was 10 times lower. The investigators suggested that the increased levels of ceruloplasmin might be related to higher cancer mortality rates found among these workers.

The immune effects of inhaled arsenic in animals were studied by Aranyi et al. (1985). Female mice exposed to arsenic trioxide aerosol for 3 hours showed a concentration-related decrease in pulmonary bactericidal activity (presumably as a result of injury to alveolar macrophages) and a corresponding concentration-related increase in susceptibility to introduced respiratory bacterial pathogens. Similar results were found when the exposure was repeated over 1- and 4-week periods. The NOAEL and LOAEL values for this study are shown in Table 3-1 and Figure 3-1.

Intratracheal studies in animals offer some support for an immune effect of inhaled inorganic arsenic. Decreases in humoral response to antigens and in several complement proteins were noted in mice given an intratracheal dose of 5.7 mg As/kg as sodium arsenite (Sikorski et al. 1989), although these changes were not accompanied by any decrease in resistance to bacterial or tumor cell challenges. Animals given an intratracheal dose of GaAs (25 mg As/kg or higher) also displayed a variety of changes in numerous immunological end points (some increased, some decreased) (Burns and Munson 1993; Sikorski et al. 1989). Whether these effects were due to a direct effect on the immune system or were secondary to the inflammatory effect of GaAs on the lung (see Section 3.2.1.2, above) is uncertain.

Organic Arsenicals. No studies were located regarding immunological and lymphoreticular effects in humans or animals after inhalation exposure to organic arsenicals.

3.2.1.4 Neurological Effects

Inorganic Arsenicals. There is evidence from epidemiological studies that inhaled inorganic arsenic can produce neurological effects. A study by Gerr et al. (2000) reported an elevated incidence of peripheral neuropathy in subjects who lived near an arsenic-using pesticide plant (13/85=15.3%; odds ratio

[OR]=5.1, p=0.004), relative to subjects who lived farther from the plant (4/118=3.4%). Concentrations of arsenic in soil and house dust were elevated (~30–300 μg As/g) for residences near the plant, according to 1993–1995 monitoring data. Studies of copper smelter workers at the ASARCO smelter in Tacoma, Washington (Feldman et al. 1979), a power station in Slovakia (Buchancová et al. 1998), and the Ronnskar smelter in Sweden (Blom et al. 1985; Lagerkvist and Zetterlund 1994) have demonstrated peripheral neurological effects in workers associated with arsenic trioxide exposure. At the ASARCO smelter, the prevalence of clinically diagnosed peripheral neuropathy was markedly higher in arsenic-exposed workers (26/61=43%) than controls (4/33=12%), and although the difference in mean nerve conduction velocities (NCV) was not statistically significant, mean peroneal motor NCV was lower in arsenic-exposed workers than controls and all 12 cases of abnormally low NCV occurred in the arsenic group (Feldman et al. 1979). In the study of 70 workers in Slovakia, the investigators described 16 cases of arsenic intoxication. Among these, 13 had signs and symptoms of sensory and motor polyneuropathy on both upper and lower extremities, 10 were diagnosed with pseudoneurasthenic syndrome, and 6 suffered from toxic encephalopathy (Buchancová et al. 1998). The average length of exposure was 22.3 years (SD ±8.4 years) and the average arsenic exposure in inhaled air ranged from 4.6 to 142.7 $\mu g/m^3$. Similar results were observed at the Ronnskar smelter, where Blom et al. (1985) reported significantly increased prevalence of workers with abnormally low NCV in the exposed group, and lower, but not statistically significant, mean NCV in five peripheral nerves. A follow-up study on the Ronnskar workers 5 years later found that the prevalence of abnormally low NCV remained significantly increased in the exposed workers, but that the decrease in mean NCV was now also statistically significant in the tibial (motor) and sural (sensory) nerves (Lagerkvist and Zetterlund 1994). Blood lead was monitored in this study as a potential confounder, but levels were low and not considered likely by the researchers to have had any influence on the results. The follow-up Ronnskar study provided enough information to estimate that mean arsenic exposure was 0.31 mg As/m^3 and lasted an average of 28 years in the exposed group, and this LOAEL is shown in Table 3-1 and Figure 3-1.

The literature also contains several case studies of workers with inhalation arsenic poisoning who developed neurological symptoms. Although these studies do not provide reliable information on exposure levels or conclusive evidence that the observed effects were related to arsenic, the findings are suggestive. Symptoms in these cases included not only indicators of peripheral neuropathy (numbness, loss of reflexes, muscle weakness, tremors) (Ide and Bullough 1988; Morton and Caron 1989), but also frank encephalopathy (hallucinations, agitation, emotional lability, memory loss) (Beckett et al. 1986; Bolla-Wilson and Bleecker 1987; Morton and Caron 1989). Both peripheral neuropathy and encephalopathy are associated with oral exposure to inorganic arsenic (see Section 3.2.2.4).

The possible association between arsenic in air and neurological effects in children has also been examined. A study by Bencko et al. (1977) reported that children of approximately 10 years of age (n=56) living near a power plant burning coal of high arsenic content showed significant hearing losses (increased threshold) compared to a control group of children (n=51) living outside the polluted area (Bencko et al. 1977). The effect was most marked at low frequencies. The precise site affected within the auditory pathway was not determined and could have been in the periphery, centrally-located, or both. A small study of children in Mexico reported a significant negative correlation between tests of verbal IQ and urinary arsenic in children (n=41) living in an urban area near a smelter complex (Calderón et al. 2001). Exposure concentrations were not available in either study.

No studies were located regarding neurological effects in animals after inhalation exposure to inorganic arsenicals. Mice given a single intratracheal dose of 200 mg/kg of GaAs displayed a decrease in overall activity 6–8 hours later, but no additional neurological evaluations were conducted on these animals (Burns and Munson 1993).

Organic Arsenicals. Data regarding neurological effects in people exposed to organic arsenic in the air are limited to a single study. The frequency of central nervous system complaints was no higher than controls in workers at a chemical factory exposed to arsanilic acid at mean concentrations up to 0.17 mg/m^3 (Watrous and McCaughey 1945). Although peripheral nerve complaints were higher in arsenic packaging workers (mean exposure=0.065 mg/m^3) than in unexposed controls, this was not the case in manufacturing workers with higher arsenic exposure (mean=0.17 mg/m^3). This suggests that the effects on the peripheral nerves in the exposed packaging workers were not due to arsenic. The reliability of these data is limited by shortcomings in the study methodology (e.g., the data might easily be biased by workers who chose not to complain about minor symptoms). No studies were located regarding neurological effects in animals after inhalation exposure to organic arsenicals.

3.2.1.5 Reproductive Effects

Inorganic Arsenicals. No studies were located regarding reproductive effects in humans after inhalation exposure to inorganic arsenicals. Reproductive performance was evaluated in female rats exposed to 0.08–20 mg As/m^3 (preliminary study) or 0.2–8 mg As/m^3 (definitive study) as As_2O_3 6 hours daily from 14 days prior to mating through gestation day 19 (Holson et al. 1999). No changes occurred in the precoital interval (time to mating), mating index (percentage of rats mated), or fertility index (percentage

of matings resulting in pregnancy). The NOAEL values for this study are shown in Table 3-1 and Figure 3-1.

Organic Arsenicals. No studies were located regarding reproductive effects in humans or animals after inhalation exposure to organic arsenicals.

3.2.1.6 Developmental Effects

Inorganic Arsenicals. Developmental effects associated with occupational and environmental exposure to airborne arsenic have been investigated in a series of studies at the Ronnskar copper smelter in northern Sweden (Nordström et al. 1978a, 1978b, 1979a, 1979b). In comparison to a northern Swedish reference population, female employees of the smelter had a significantly increased incidence of spontaneous abortion (Nordström et al. 1979a), and their children had a significantly increased incidence of congenital malformations (Nordström et al. 1979b) and significantly decreased average birth weight (Nordström et al. 1978a). Increased incidence of spontaneous abortion and decreased average birth weight of children were also found in populations living in close proximity to the smelter (Nordström et al. 1978a, 1978b, 1979b). While these data are suggestive of developmental effects associated with occupational and environmental exposure from the smelter, the reported effects are not large, the analyses include only limited consideration of potential confounders (e.g., smoking), and there are no data relating the apparent effects specifically to arsenic exposure.

Ihrig et al. (1998) conducted a case-control study of stillbirths in the vicinity of a Texas arsenic pesticide factory that included estimation of environmental arsenic exposures using atmospheric dispersion modeling and multiple regression analysis considering arsenic exposure, race/ethnicity, maternal age, median income, and parity as explanatory variables. There was a statistically significant increase in the risk of stillbirth in the highest exposure category (>100 ng As/m^3, midpoint=682 ng/m^3). Further analysis showed that this increase in risk was limited to people of Hispanic descent, who the researchers speculated may be an especially sensitive population due to a genetic impairment in folate metabolism. Interpretation of this study is limited by small numbers of cases and controls in the high exposure group, lack of data on smoking, potential confounding exposures to other chemicals from the factory, and failure to take into account previous years of deposition in the exposure estimates.

Arsenic has been shown to produce developmental effects by inhalation exposure in laboratory animals, although it is unclear whether or not the effects occur only at maternally toxic doses. Mice exposed to

22 mg As/m^3 (as As_2O_3) for 4 hours on days 9–12 of gestation had serious developmental effects (significant increases in the percentage of dead fetuses, skeletal malformations, and the number of fetuses with retarded growth), while those exposed to 2.2 mg As/m^3 had only a 10% decrease in average fetal body weight, and those exposed to 0.20 mg As/m^3 had no effects (Nagymajtényi et al. 1985). The study was limited by failure to quantify malformations on a litter basis, discuss the nature and severity of the observed malformations, or report on the occurrence of maternal effects. No increases in fetal resorptions, fetal mortality, or malformations, and no decreases in fetal body weight occurred when rats were exposed to 0.2–8 mg As/m^3 (as As_2O_3), 6 hours daily from 14 days prior to mating through gestation day 19 (Holson et al. 1999). At the 8 mg/m^3 exposure level, toxicity was observed in the dams, including rales, a dried red exudate at the nose, and lower gains in net body weight than controls. In a preliminary dose-range study, there was a marked significant increase in postimplantation loss (primarily early resorptions) and consequent marked significant decrease in viable fetuses per litter at 20 mg As/m^3, a concentration that also produced severe maternal effects including mortality (Holson et al. 1999).

The NOAEL and LOAEL values for increased risk of stillbirth in humans identified by Ihrig et al. (1998) and those for developmental effects in rodents found by Nagymajtényi et al. (1985) and Holson et al. (1999) are shown in Table 3-1 and Figure 3-1.

Organic Arsenicals. No studies were located regarding developmental effects in humans or animals after inhalation exposure to organic arsenicals.

3.2.1.7 Cancer

Inorganic Arsenicals. There is convincing evidence from a large number of epidemiological studies that inhalation exposure to inorganic arsenic increases the risk of lung cancer. Most studies involved workers exposed primarily to arsenic trioxide dust in air at copper smelters (Axelson et al. 1978; Brown and Chu 1982, 1983a, 1983b; Enterline and Marsh 1982; Enterline et al. 1987a, 1987b, 1995; Ferreccio et al. 1996; Järup and Pershagen 1991; Järup et al. 1989; Lee and Fraumeni 1969; Lee-Feldstein 1983, 1986; Lubin et al. 2000; Mazumdar et al. 1989; Pinto et al. 1977, 1978; Sandstrom et al. 1989; Viren and Silvers 1999; Wall 1980; Welch et al. 1982) and mines (Liu and Chen 1996; Qiao et al. 1997; Taylor et al. 1989; Xuan et al. 1993), but increased incidence of lung cancer has also been observed at chemical plants where exposure was primarily to arsenate (Bulbulyan et al. 1996; Mabuchi et al. 1979; Ott et al. 1974; Sobel et al. 1988). In addition, several studies suggest that residents living near smelters or arsenical chemical plants may also have increased risk of lung cancer (Brown et al. 1984; Cordier et al. 1983; Matanoski et

al. 1981; Pershagen 1985), although the increases are small and are not clearly detectable in all cases (e.g., Frost et al. 1987). The strongest evidence that arsenic is responsible for the observed lung cancer comes from quantitative dose-response data relating specific arsenic exposure levels to lung cancer risk. These data are available for arsenic-exposed workers at the ASARCO copper smelter in Tacoma, Washington (Enterline and Marsh 1982; Enterline et al. 1987a, 1995; Mazumdar et al. 1989), the Anaconda copper smelter in Montana (Lee-Feldstein 1986; Welch et al. 1982), eight other U.S. copper smelters (Enterline et al. 1987b), and the Ronnskar copper smelter in Sweden (Järup and Pershagen 1991; Järup et al. 1989). A common limitation of these studies is confounding exposure to other chemicals, such as sulfur dioxide, and cigarette smoking.

Enterline and Marsh (1982) reported a significant increase in respiratory cancer mortality (standard mortality ratio [SMR]=189.4) based on 104 observed respiratory cancer deaths and only 54.9 expected over the years 1941–1976 in a cohort of 2,802 male workers employed for ≥1 year between 1940 and 1964 at the ASARCO smelter. When the cohort was separated into low and high arsenic exposure groups, with mean estimated time-weighted average arsenic exposures of 0.054 and 0.157 mg As/m^3, respectively (based on work history, historical urinary arsenic measurements, and an experimentally derived relationship between urinary and inhaled arsenic), respiratory cancer mortality was significantly increased in both groups in a concentration-related fashion (SMR=227.7 and 291.4 in the low and high groups, respectively). Enterline et al. (1987a) re-analyzed these data using improved exposure estimates that incorporated historical measurements of arsenic in the ambient air and personal breathing zone of workers. Respiratory cancer mortality was significantly increased in a concentration-related fashion in the low (SMR=213.0), medium (SMR=312.1), and high (SMR=340.9) arsenic exposure groups, which had mean estimated time-weighted average arsenic exposures of 0.213, 0.564, and 1.487 mg As/m^3, respectively. An alternative analysis of these data by Mazumdar et al. (1989) produced similar results. Enterline et al. (1995) extended the mortality follow-up from 1976 to 1986, but reported findings similar to the earlier study in a less thorough analysis. The CEL from Enterline et al. (1987a), the most complete analysis of the ASARCO cohort with the best exposure estimates, is presented in Table 3-1 and Figure 3-1.

Respiratory cancer mortality was significantly increased (SMR=285) based on 302 observed respiratory deaths between 1938 and 1977 in a cohort of 8,045 white male workers employed for at least 1 year between 1938 and 1956 at the Anaconda smelter (Lee-Feldstein 1986). When workers were categorized according to cumulative arsenic exposure and date of hire, lung cancer mortality was significantly increased in all groups hired between 1925 and 1947. Workers in the lowest cumulative exposure group

(<10 mg-mo/m^3) were reported to have had <2 years of exposure at an average arsenic concentration of 0.38 mg/m^3. An alternative analysis of a subset of the Anaconda cohort (n=1,800, including all 277 employees with heavy arsenic exposure and 20% of the others) that included information on smoking and other occupational exposures was performed by Welch et al. (1982). This analysis showed that lung cancer mortality increased with increasing time-weighted average arsenic exposure, with a small nonsignificant increase in the low group (SMR=138) exposed to 0.05 mg/m^3 and significant increases in the medium (SMR=303), high (SMR=375), and very high (SMR=704) groups exposed to 0.3, 2.75, and 5.0 mg/m^3, respectively. Cohort members were more likely to be smokers than U.S. white males, but smoking did not differ among the arsenic exposure groups. Exposure-response analysis of smokers was similar to the analysis based on the full subcohort, while analysis of nonsmokers (limited by small group sizes) also showed a similar pattern, but with lower SMRs. In a followup analysis of the same cohort, Lubin et al. (2000) re-weighted the exposure concentrations based on duration and time of exposure and re-evaluated the effects of exposure. Relative risks for respiratory cancer increased with increasing duration in each arsenic exposure area (light, medium, and heavy) after adjustment for duration in the other two exposure areas. SMRs were significantly elevated following exposure to 0.58 mg/m^3 (medium; SMR=3.01, 95% CI=2.0–4.6) or 11.3 mg/m^3 (high; SMR=3.68, 95% CI=2.1–6.4) for 10 or more years, and following exposure to 0.29 mg/m^3 (low; SMR=1.86, 95% CI=1.2–2.9) for 25 or more years. The CELs from the analyses of the Anaconda cohort are presented in Table 3-1 and Figure 3-1.

Enterline et al. (1987b) studied the mortality experience from 1949 to 1980 of a cohort of 6,078 white males who had worked for 3 years or more between 1946 and 1976 at one of eight U.S. copper smelters in Arizona, Utah, Tennessee, and Nevada. Lung cancer mortality was significantly increased only in the Utah smelter (SMR=226.7), which had the highest average arsenic exposure concentration (0.069 mg/m^3 vs. 0.007–0.013 mg/m^3 in the other smelters) and also contributed the largest number of cohort members (n=2,288 vs. 189–965 from the other smelters). A nested case-control study showed that arsenic exposure and cigarette smoking were significant risk factors for lung cancer in the smelter workers. Smoking was lower in the Utah smelter workers than in the other smelter workers, but still higher than in the referent Utah population, suggesting that the risk attributable to arsenic in this study population is somewhat lower than indicated by the SMR reported above. The CEL from this study is presented in Table 3-1 and Figure 3-1.

Järup et al. (1989) reported significantly increased lung cancer mortality (SMR=372, 95% confidence interval [CI]=304–450) based on 106 lung cancer deaths in a cohort of 3,916 male workers employed for ≥3 months between 1928 and 1967 at the Ronnskar smelter and followed for mortality through 1981.

Workers were separated into low, medium, and high arsenic exposure groups with mean time-weighted average exposure estimates of 0.05, 0.2, and 0.4 mg/m^3, respectively. Lung cancer mortality was significantly increased in all three exposure groups in a concentration-related fashion (SMR=201, 353, and 480, respectively). A nested case-control analysis of 102 lung cancer cases and 190 controls from the cohort showed that lung cancer risk increased with increasing arsenic exposure in nonsmokers, light smokers, and heavy smokers (Järup and Pershagen 1991). The results demonstrated that arsenic is a risk factor for lung cancer in the smelter workers, but also suggested a greater-than-additive interaction between smoking and arsenic exposure. In this analysis, in contrast to the cohort study, lung cancer risk due to arsenic was increased only in the higher arsenic-exposure groups. Potential explanations for this difference between the cohort and case-control analyses include a higher proportion of smokers in the smelter workers than in the regional referent population in the cohort study, and limited power to detect increased risk in the case-control study due to small group sizes in the dose-response analysis. The CELs from both the cohort and case-control studies are presented in Table 3-1 and Figure 3-1.

Several researchers have examined the histological cell types of lung cancer (epidermoid carcinoma, small cell carcinoma, adenocarcinoma) in arsenic-exposed workers (e.g., Axelson et al. 1978; Newman et al. 1976; Pershagen et al. 1987; Qiao et al. 1997; Wicks et al. 1981). Although the incidence of the various cell types varied from population to population, all studies found an increase in several tumor types. This indicates that arsenic does not specifically increase the incidence of one particular type of lung cancer.

The studies of the ASARCO cohort (Enterline and Marsh 1982; Enterline et al. 1987a, 1995) noted a supralinear exposure-response relationship (i.e., steeper at lower doses) between arsenic exposure and lung cancer mortality. Hertz-Picciotto and Smith (1993) extended this observation to several other occupationally exposed cohorts with quantitative exposure information. The authors suggest that neither toxicokinetic mechanisms nor confounding from age, smoking, or other workplace carcinogens that differ by exposure level are likely explanations for the curvilinearity. Plausible explanations offered include: (1) synergism (with smoking), which varies in magnitude according to the level of arsenic exposure, (2) long-term survivorship at higher exposures among the healthier, less susceptible individuals, and (3) exposure estimate errors that were more prominent at higher-exposure levels as a result of past industrial hygiene sampling or worker protection practices.

Quantitative risk estimates for inhaled inorganic arsenic have been derived using the exposure-response data. EPA derived a unit risk estimate (the excess risk of lung cancer associated with lifetime exposure to

1 μg/m^3) of 4.3x10^{-3} per (μg/m^3) based on the dose-response relationships between arsenic exposure and excess lung cancer mortality in workers at the Anaconda smelter in Montana (Brown and Chu 1982, 1983a, 1983b; Lee-Feldstein 1983; and an unpublished paper by Higgins and associates) and the ASARCO smelter in Tacoma, Washington (Enterline and Marsh 1982; EPA 1984a; IRIS 2007). In some cases, calculations of exposure, as well as the procedures for generating quantitative risk estimates, are quite complex and the interested reader is referred to the EPA documents (EPA 1981c, 1984a, 1987e, 1996b; IRIS 2007) for a detailed description. Viren and Silvers (1994) re-evaluated the unit risk estimate using the same methods as EPA, but incorporating updated results from the ASARCO smelter (Enterline et al. 1987a; Mazumdar et al. 1989) and the findings from the Swedish smelter (Järup et al. 1989). Their analysis yielded a revised unit risk of 1.28x10^{-3} per (μg/m^3) that, when pooled with the earlier estimate from the Montana smelter cohort, yielded a composite unit risk of 1.43x10^{-3} per (μg/m^3). This unit risk estimate is a factor of 3 smaller than the EPA's current estimate of 4.3x10^{-3} per (μg/m^3). Figure 3-1 shows the air concentrations that correspond to excess lifetime cancer risks of 10^{-4}–10^{-7} based on the EPA unit risk estimate.

There have been occasional reports of other types of cancer (i.e., nonrespiratory cancer) potentially associated with inhalation exposure to inorganic arsenic, but there is no strong evidence for any of them. For example, Enterline et al. (1995) found significantly increased mortality due to cancer of the large intestine and bone cancer in the ASARCO cohort. However, neither cancer showed any relation to cumulative arsenic exposure, and the purported increase in bone cancer risk was based on a very small number of observations. Pesch et al. (2002) reported an increase in nonmelanoma skin cancers resulting from exposure from a Slovakian coal-burning power plant, but exposure levels associated with the lesions were not presented. Bencko et al. (2005) also reported an increase in the incidence of nonmelanoma skin cancer among workers of a power plant burning coal of a high arsenic content and in the population living in the vicinity of the power plant. Bulbulyan et al. (1996) reported an increase in risk of stomach cancer among workers exposed to the highest average arsenic concentrations at a Russian fertilizer plant, but this finding, which was based on a small number of observations and was only marginally statistically significant, was confounded by exposure to nitrogen oxides, which were more convincingly associated with stomach cancer in this study. Wingren and Axelson (1993) reported an association between arsenic exposure and stomach and colon cancer in Swedish glass workers, but this result was confounded by concomitant exposure to other metals. Lee-Feldstein (1983) observed a small, marginally significant increase in digestive tract cancer (SMR=125) in one study of the Anaconda cohort, but this was not found in other studies of this cohort (Lee and Fraumeni 1969; Lee-Feldstein 1986; Welch et al. 1982). Wulff et al. (1996) observed an apparent increase in the risk of childhood cancer (all types combined) in the

population living within 20 km of the Ronnskar smelter, but the apparent increase was based on a small number of cases (13 observed vs. 6.7 expected) and was not statistically significant, and exposure to arsenic was confounded by exposure to lead, copper, cadmium, sulfur dioxide, and possibly other emissions such as nickel and selenium. A retrospective study of deaths due to unspecified types of malignancies among workers of power plants found no significant differences in death rate between two groups whose exposure levels to arsenic had a difference of one order of magnitude (Bencko et al. 1980). However, the mean age of those deceased due to cancer in the high-exposure group was 55.9 years compared to 61.2 years in the low-exposure group, and this difference was statistically significant ($p<0.05$). Also, when the workers were stratified by exposure-duration, there was a significantly higher frequency of tumors in the high-exposure group after shorter employment periods (<5 or 6–10 years) than after a longer employment period (≥11 years). No information was provided regarding specific types of cancer. Various case reports have implicated occupational arsenic exposure as a potential contributing factor in workers who developed sinonasal cancer (Battista et al. 1996), hepatic angiosarcoma (Tsai et al. 1998a), and skin cancer (Cöl et al. 1999; Tsuruta et al. 1998), but provide no proof that inhaled arsenic was involved in the etiology of the observed tumors. Wong et al. (1992) found no evidence that environmental exposure to airborne arsenic produced skin cancer in residents living near the Anaconda smelter or an open pit copper mine.

No studies were located regarding cancer in animals after inhalation exposure to inorganic arsenicals, although several intratracheal instillation studies in hamsters have provided evidence that both arsenite and arsenate can increase the incidence of lung adenomas and/or carcinomas (Ishinishi et al. 1983; Pershagen and Björklund 1985; Pershagen et al. 1984; Yamamoto et al. 1987). These data support the conclusion that inhalation of arsenic may lead to lung cancer in humans.

Organic Arsenicals. No studies were located regarding cancer effects in humans or animals after inhalation exposure to organic arsenicals.

3.2.2 Oral Exposure

There are a large number of studies in humans and animals on the toxic effects of ingested arsenic. In humans, most cases of toxicity have resulted from accidental, suicidal, homicidal, or medicinal ingestion of arsenic-containing powders or solutions or by consumption of contaminated food or drinking water. In some cases, the chemical form is known (e.g., the most common arsenic medicinal was Fowler's solution, which contained 1% potassium arsenite or arsenic trioxide), but in many cases (e.g., exposures through

drinking water), the chemical form is not known. In these cases, it is presumed that the most likely forms are either inorganic arsenate [As(+5)], inorganic arsenite [As(+3)], or a mixture. Table 3-3 and Figure 3-3 summarize a number of studies that provide reliable quantitative data on health effects in humans and animals exposed to inorganic arsenicals by the oral route. Similar data for MMA, DMA, and roxarsone are listed in Tables 3-4, 3-5, and 3-6, and shown in Figures 3-4, 3-5, and 3-6, respectively. All exposure data are expressed as milligrams of arsenic (as the element) per kilogram body weight per day (mg As/kg/day). These studies and others that provide useful qualitative information are summarized below.

3.2.2.1 Death

Inorganic Arsenicals. There are many case reports of death in humans due to ingestion of high doses of arsenic. In nearly all cases, the most immediate effects are vomiting, diarrhea, and gastrointestinal hemorrhage, and death may ensue from fluid loss and circulatory collapse (Levin-Scherz et al. 1987; Saady et al. 1989; Uede and Furukawa 2003). In other cases, death may be delayed and result from the multiple tissue injuries produced by arsenic (Campbell and Alvarez 1989). Some accounts of fatal arsenic poisoning describe both gastrointestinal effects soon after ingestion and extensive damage to multiple organ systems prior to death (Quatrehomme et al. 1992). A precise estimate of the ingested dose is usually not available in acute poisonings, so quantitative information on lethal dose in humans is sparse. The lethal doses ranged from 22 to 121 mg As/kg in four cases where known amounts were ingested as a single bolus (Civantos et al. 1995; Hantson et al. 1996; Levin-Scherz et al. 1987; Quatrehomme et al. 1992). Two people in a family of eight died from ingestion of water containing about 110 ppm of arsenic for a week (Armstrong et al. 1984). This corresponded to a dose of about 2 mg As/kg/day. Based on a review of clinical reports in the older literature, Holland (1904) estimated the minimum lethal dose to be about 130 mg (also about 2 mg/kg). A similar estimate of 70–180 mg (about 1–3 mg/kg) was provided by Vallee et al. (1960). Death due to chronic arsenic exposure has been reported at lower concentrations. Five children between the ages of 2 and 7 years died from late sequelae of chronic arsenic poisoning after drinking contaminated water throughout their lives at estimated average doses of 0.05–0.1 mg As/kg/day (Zaldívar and Guillier 1977). A 22-year-old man with chronic arsenical dermatosis died from arsenic-related effects after lifetime exposure to an estimated average dose of 0.014 mg As/kg/day in the drinking water (Zaldívar et al. 1981). Systematic studies of lethality from chronic exposure attributable to increased risk of cardiovascular disease or cancer are discussed below in Sections 3.2.2.2 and 3.2.2.7, respectively.

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Human	1 wk (W)				2 (death)	Armstrong et al. 1984 NS	
2	Human	once (IN)				121 M (death)	Civantos et al. 1995 As(+5)	
3	Human	once (IN)				108 M (death)	Hantson et al. 1996 As(+3)	
4	Human	once (IN)				22 M (death)	Levin-Scherz et al. 1987 As(+3)	
5	Human	once (IN)				93 M (death)	Quatrehomme et al. 1992 As(+3)	
6	Rat (wild Norway)	once (G)				104 (LD50)	Dieke and Richter 1946 As(+3)	
7	Rat (Sherman)	once (G)				112 F (LD50)	Gaines 1960 As(+5) calcium arsenate	
8	Rat (Sherman)	once (G)				44 F (LD50)	Gaines 1960 As(+3)	
9	Rat (Sherman)	once (G)				175 F (LD50)	Gaines 1960 As(+5) lead arsenate	
10	Rat (Sprague-Dawley)	once (GW)				15 M (LD50)	Harrisson et al. 1958 As(+3)	

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
11	Rat (Sprague-Dawley)	once (F)				145 M (LD50)	Harrisson et al. 1958 As(+3)	
12	Rat (CD)	once Gd 9 (GW)				23 F (7/25 dams died)	Stump et al. 1999 As(+3)	
13	Mouse (Swiss-Webster)	once (GW)				39 M (LD50)	Harrisson et al. 1958 As(+3)	
14	Mouse (C57H46)	once (GW)				26 M (LD50)	Harrisson et al. 1958 As(+3)	
15	Mouse (Dba2)	once (GW)				32 M (LD50)	Harrisson et al. 1958 As(+3)	
16	Mouse (C3H)	once (GW)				26 M (LD50)	Harrisson et al. 1958 As(+3)	
17	Mouse (ddY)	once (GW)				26 M (LD50)	Kaise et al. 1985 As(+3)	
18	Rabbit (New Zealand)	Gd 6-18 1 x/d (GW)				1.49 F (7/20 dams died)	Nemec et al. 1998 As(+5)	

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Systemic								
19	Human	1 wk (W)	Gastro		0.2 (vomiting, diarrhea, abdominal pain)	2 M (diffuse inflammation of the GI tract)	Armstrong et al. 1984 NS	
			Hemato			0.2 (pancytopenia, leukopenia)		
			Hepatic			0.4 (hepatitis)		
			Renal			0.2 (nephropathy)		
			Ocular		0.2 (periorbital swelling)			
20	Human	once (IN)	Resp			121 M (respiratory distress, lung hemmorhage and edema)	Civantos et al. 1995 As(+5)	
			Cardio			121 M (hypotension, ventricular fibrillation, cardiac arrest)		
			Gastro			121 M (ulceration of upper gastrointestinal tract)		

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
21	Human	once (IN)	Cardio			19 F (tachycardia)	Cullen et al. 1995 As (+5)	
			Gastro			19 F (profuse vomiting and diarrhea)		
			Hemato	19 F				
			Hepatic	19 F				
			Renal	19 F				
22	Human	once (NS)	Resp			8 M (hemorrhagic bronchitis, pulmonary edema)	Fincher and Koerker 1987 As(+3)	
			Cardio			8 M (hypotension, tachycardia, massive cardiomegaly)		
			Gastro			8 M (gastrointestinal bleeding)		
			Hemato			8 M (hemolysis)		
			Musc/skel			8 M (marked atrophy of distal muscle groups)		
			Renal			8 M (acute renal failure)		
			Dermal		8 M (truncal macular rash)			
23	Human	1 or 2 x (W)	Gastro		0.05 (occasional nausea, diarrhea, and abdominal cramps)		Franzblau and Lilis 1989 As(+3) As(+5)	

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
24	Human	once (W)	Gastro			120 M (vomiting and diarrhea)	Goebel et al. 1990 NS	
			Renal			120 M (anuria)		
			Dermal		120 M (hyperkeratosis)			
25	Human	once (IN)	Gastro		2 F (vomiting)		Hantson et al. 1996 As(+3)	
			Hepatic		2 F (slight increase in serum bilirubin)			
			Renal		2 F (altered renal function tests)			
26	Human	once (IN)	Gastro			13 M (frequent vomiting, diarrhea)	Kamijo et al. 1998 As(+3)	
			Hepatic			13 M (large increase in serum bilirubin, ALT, AST, LDH)		
			Dermal		13 M (erythematous eruption)			
			Ocular		13 M (constricted vision)			

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
27	Human	once (IN)	Resp			22 M (tachypnea, respiratory failure)	Levin-Scherz et al. 1987 As(+3)	
			Cardio			22 M (cyanosis, hypotension, tachycardia, ventricular fibrillation)		
			Gastro			22 M (abdominal pain, nausea, diarrhea, massive vomiting, dysphagia, hemorrhage)		
			Hepatic			22 M (large increase in serum AST and LDH)		
			Renal			22 M (large increase in serum creatinine and BUN indicating acute renal failure)		
28	Human	once pregnancy wk 30 (IN)	Cardio			6 F (hypotension, rapid pulse)	Lugo et al. 1969 As(+3)	
			Gastro			6 F (abdominal pain, vomiting)		
			Hemato		6 F (high leukocyte count, low hematocrit)			
			Renal			6 F (acute renal failure)		

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form	Comments
29	Human	2-3 wk (F)	Resp		0.05	(sore throat, rhinorrhea, cough, sputum)			Mizuta et al. 1956 As(+5)	
			Cardio				0.05	(abnormal electrocardiogram)		
			Gastro				0.05[b]	(nausea, vomiting, diarrhea, occult blood in feces and gastric and duodenal juice)		
			Hemato		0.05	(mild anemia, leukopenia)				
			Musc/skel		0.05	(tender calf muscle)				
			Hepatic		0.05	(mild hepatomegaly, impaired liver function, degenerative lesions)				
			Renal	0.05						
			Dermal		0.05	(pigmentation, itching, desquamation, exanthema)				
			Ocular		0.05	(edema of eyelids, conjunctivitis, central scotoma, neuro-retinitis)				

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
30	Human	once (IN)	Resp	11 M		43 M (shortness of breath, decreased oxygen saturation)	Moore et al. 1994a As(+3)	
			Cardio	11 M		43 M (hypotension, asystolic cardiac arrest)		
			Gastro			11 M (profuse diarrhea and vomiting, severe abdominal pain)		
			Hemato	43 M				
			Renal		11 M (increased serum creatinine)	43 M (acute renal failure)		
31	Human	once (IN)	Resp			93 M (pulmonary edema)	Quatrehomme et al. 1992 As(+3)	
			Gastro			93 M (ulcero-necrotic hemorrhagic gastritis)		
			Hepatic			93 M (hepatomegaly, diffuse fatty degeneration)		
			Renal			93 M (glomerular congestion)		
			Dermal			93 M (dermoepidermic separation)		

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
32	Monkey (Rhesus)	13 d 1 x/d (IN)	Gastro	3		6 (vomiting, unformed stool, "loss of condition")	Heywood and Sortwell 1979 As(+5)	
			Hepatic	3	6 (decreased liver glycogen, vacuolation of hepatocytes)			
			Renal	3	6 (dilation of proximal tubules)			
33	Rat (Wistar-Barby)	4-14 d 5 d/wk 1 x/d (G)	Cardio	2 F	11 F (decreased vasoreactivity)		Bekemeier and Hirschelmann 1989 As(+3)	
			Gastro	2 F		11 F (diarrhea, bloody stools)		
34	Rat (Sprague-Dawley)	2 x (GW)	Resp	14 F			Brown and Kitchin 1996 As(+3)	
			Hepatic		0.9 F (slight increased ornithine decarboxylase and heme oxygenase activity in liver)			
			Dermal	14 F				
35	Rat (Sprague-Dawley)	2 x (GW)	Hepatic	8 F	24 F (increased heme oxygenase activity in liver)		Brown et al. 1997c As(+5)	

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
36	Rat (Sprague-Dawley)	1 x/d 15 d (G)	Bd Wt	10 M	20 M (20-25% decreased body weight)		Rodriguez et al. 2001 As(+3)	
37	Rat (CD)	once Gd 9 (GW)	Bd Wt	15 F	23 F (decreased body weight gain)		Stump et al. 1999 As(+3)	
38	Mouse (CD-1)	Gd 6-15 1 x/d (GW)	Bd Wt	12 F	24 F (decreased body weight gain during gestation)		Nemec et al. 1998 As(+5)	
39	Mouse (B6C3F1)	1 or 4 d 1 x/d (GW)	Hemato	3 M	6 M (decreased polychromatic erythrocytes in bone marrow)		Tice et al. 1997 As(+3)	
40	Gn Pig	1 x/d 8 d (G)	Cardio		3.8 M (prolongation of QT interval)		Chiang et al. 2002 As2O3	
41	Rabbit (New Zealand)	Gd 6-18 1 x/d (GW)	Bd Wt	0.37 F	1.49 F (loss of body weight during treatment during gestation)		Nemec et al. 1998 As(+5)	
Neurological								
42	Human	1 wk (W)				2 (encephalopathy, peripheral neuropathy)	Armstrong et al. 1984 NS	

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
43	Human	once (IN)				121 M (confusion, brain edema)	Civantos et al. 1995 As(+5)	
44	Human	once (IN)				19 F (lethargy)	Cullen et al. 1995 As (+5)	
45	Human	once (NS)				8 M (severe, persistent encephalopathy and peripheral neuropathy)	Fincher and Koerker 1987 As(+3)	
46	Human	once (W)				120 M (severe polyneuropathy)	Goebel et al. 1990 NS	
47	Human	once (IN)				216 M (peripheral neuropathy)	Hantson et al. 1996 As(+3)	
48	Human	once (IN)				13 M (peripheral neuropathy)	Kamijo et al. 1998 As(+3)	
49	Human	once (IN)				22 M (agitation, disorientation, paranoia, violent reactions)	Levin-Scherz et al. 1987 As(+3)	
50	Human	2-3 wk (F)				0.05 (hypesthesia in legs, abnormal patellar reflex)	Mizuta et al. 1956 As(+5)	
51	Human	once (IN)		43 M			Moore et al. 1994a As(+3)	

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
52	Human	once (IN)				93 M (encephalopathy)	Quatrehomme et al. 1992 As(+3)	
53	Monkey (Rhesus)	13 d 1 x/d (IN)		3		6 (marked salivation, uncontrolled head shaking)	Heywood and Sortwell 1979 As(+5)	
54	Rat (Sprague-Dawley)	1 x/d 15 d (G)		10 M	20 M (altered spontaneous locomotor activity)		Rodriguez et al. 2001 As(+3)	
55	Rabbit (New Zealand)	Gd 6-18 1 x/d (GW)		0.37 F		1.49 F (prostration, ataxia)	Nemec et al. 1998 As(+5)	
Developmental								
56	Human	once pregnancy wk 30 (IN)				6 (severe pulmonary hemorrhage that may have contributed to death in premature neonate)	Lugo et al. 1969 As(+3)	
57	Rat (CD)	once Gd 9 (GW)		15		23 (increased post-implantation loss and decreased viable fetuses)	Stump et al. 1999 As(+3)	

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form	Comments
58	Mouse (CD-1)	once Gd 8-15 (GW)		11		23	(increased fetal mortality, exencephaly)	Baxley et al. 1981 As(+3)	
59	Mouse (CD-1)	once Gd 7-15 (GW)				48	(increased fetal death, decreased fetal weight, gross and skeletal malformations)	Hood et al. 1978 As(+5)	
60	Mouse (CD-1)	Gd 6-15 1 x/d (GW)		12		24	(increased resorptions per litter, decreased live fetuses per litter, decreased mean fetal weight)	Nemec et al. 1998 As(+5)	
61	Hamster (Lak:LVG [SYR])	once Gd 8-12 (GW)		11		14	(increased fetal mortality, decreased fetal weight)	Hood and Harrison 1982 As(+3)	
62	Rabbit (New Zealand)	Gd 6-18 1 x/d (GW)		0.37		1.49	(increased resorptions per litter, decreased live fetuses per litter)	Nemec et al. 1998 As(+5)	

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Cancer								
63	Mouse C3H	10 d (W)				9.55 M (CEL: liver and adrenal tumors)	Waalkes et al. 2003 As(+3)	
						19.13 F (CEL: ovarian and lung tumors)		
INTERMEDIATE EXPOSURE								
Systemic								
64	Human	3 mo (W)	Gastro			0.1 (severe nausea, diarrhea, pain, cramps, vomiting, traces of blood in stool)	Franzblau and Lilis 1989 As(+3) As(+5)	
			Hemato			0.1 (anemia, leukopenia)		
			Hepatic			0.1 (large increased AST and ALT)		
			Dermal		0.1 (diffuse erythematous and scaly rash)			
			Ocular		0.1 (swelling and irritation of the eyes, impaired peripheral vision)			
65	Human	0.5-14 yr (W)	Dermal			0.05 (hyperpigmentation with keratosis, possibly pre-cancerous)	Huang et al. 1985 NS	

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
66	Human	4 mo (W)	Gastro			0.06 F (nausea, vomiting, diarrhea)	Wagner et al. 1979 NS	
			Hemato			0.06 F (anemia, leukopenia, erythroid hyperplasia of bone marrow)		
			Dermal			0.06 F (persistent extensive hyperkeratosis of palms and soles)		
			Bd Wt			0.06 F (40 lb weight loss)		
67	Rat (Wistar-Barby)	4 wk 5 d/wk 1 x/d (GW)	Cardio		11 F (decreased vasoreactivity)		Bekemeier and Hirschelmann 1989 As(+3)	
68	Rat (Sprague-Dawley)	6 wk (W)	Renal		4.7 M (increased relative kidney weight, impaired renal mitochondrial respiration, ultrastructural changes in proximal tubule)		Brown et al. 1976 As(+5)	
			Bd Wt	9.4 M	10.9 M (decreased body weight gain)			

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
69	Rat (Wistar)	1 x/d 28 d (G)	Bd Wt	0.14 F			Chattopadhyay et al. 2001 As(+3)	
70	Rat (CD)	6 wk (W)	Hepatic	3 M	6 M (ultrastructural changes in hepatocytes, impaired liver mitochondrial respiration)		Fowler et al. 1977 As(+5)	
			Bd Wt	6 M		12 M (final body weight 28% lower than controls)		
71	Rat (CD)	14 pmd- Gd 19 7 d/wk 6 hr/d (GW)	Gastro	4 F		8 F (stomach adhesions, eroded luminal epithelium in the stomach)	Holson et al. 2000 As(+3)	
			Hepatic	2 F	4 F (increased liver weight)			
			Renal	4 F	8 F (increased kidney weight)			
			Bd Wt	4 F	8 F (decreased body weight gain)			
72	Rat (NS)	16 wk (W)	Hemato		0.92 M (decreased erythrocyte and leukocyte numbers)		Kannan et al. 2001 As(+3)	
			Hepatic	2.3 M				

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
73	Rat (Sprague-Dawley)	4 wk (W)	Hemato	0.12	0.3 (increased platelet aggregation)		Lee et al. 2002 As(+3)	
74	Rat (NS)	1 x/d 30 d (G)	Endocr		2.3 M (decreased islet cells in pancreas, increased pancreatic SOD and catalase)		Mukherjee et al. 2003 As2O3	
75	Rat (Wistar)	1 x/d 5 d/wk 12 wk (G)	Resp	19 M			Schulz et al. 2002 As(+3)	
			Renal	19 M				
			Bd Wt	9.5 M	19 M (~17% decreased body weight gain)			
76	Mouse (C57BL)	6 wk (W)	Hepatic	5 M	10 M (ultrastructural changes in hepatocytes, impaired liver mitochondrial respiration)		Fowler and Woods 1979 As(+5)	
			Bd Wt	5 M	10 M (decreased body weight gain)			

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
77	Mouse (C57BL/6 B6)	14 wk (W)	Hepatic	25 M			Kerkvliet et al. 1980 As(+5)	
			Renal	25 M				
78	Gn Pig (NS)	16 wk (W)	Hemato		0.69 M (decreased erythrocyte number and leukocyte number, decreased ALAD levels)		Kannan et al. 2001 As(+3)	
			Hepatic		0.69 M (increased ALAS activity)			
79	Dog (Beagle)	26 wk ad lib (F)	Hemato	1.9 F			Neiger and Osweiler 1989 As(+3)	
			Hepatic		0.8 F (mild increased serum ALT/AST)			
			Renal	1.9 F				
			Bd Wt	0.8 F	1.5 F (decreased body weight gain)	1.9 F (25% decrease in body weight)		
Immuno/ Lymphoret								
80	Mouse (C57BL/6 B6)	14 wk (W)		25 M			Kerkvliet et al. 1980 As(+5)	
Neurological								
81	Human	3 mo (W)				0.1 (paresthesia of hands and feet; confusion, disorientation and mental sluggishness)	Franzblau and Lilis 1989 As(+3) As(+5)	

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
82	Human	4 mo (W)				0.06 F (weakness, paresthesia)	Wagner et al. 1979 NS	
83	Rat (NS)	16 wk (W)		0.92 M	2.3 M (decreased brain neurotransmitter levels)		Kannan et al. 2001 As(+3)	
84	Rat (Wistar)	1 x/d 5 d/wk 12 wk (G)		19 M			Schulz et al. 2002 As(+3)	
85	Gn Pig (NS)	16 wk (W)		0.69 M	1.7 M (changes in brain neurotransmitter levels)		Kannan et al. 2001 As(+3)	
Reproductive								
86	Rat (Wistar)	1 x/d 28 d (G)			0.14 F (changes in uterine and ovarian weights, decreased estradiol)		Chattopadhyay et al. 2001 As(+3)	
87	Rat (CD)	14 pmd- Gd 19 7 d/wk 6 hr/d (GW)		8 F			Holson et al. 2000 As(+3)	
88	Mouse (CD)	3 gen (W)				1 (decreased litter size)	Schroeder and Mitchener 1971 As(+3)	

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Developmental								
89	Rat (CD)	14 pmd- Gd 19 7 d/wk 6 hr/d (GW)		4	8 (decreased fetal body weight, increased skeletal variations)		Holson et al. 2000 As(+3)	
90	Rat (Sprague-Dawley)	Gd 15 or pnd 1-4 mo (W)			2.93 M (impaired performance in postnatal neurobehavioral tests)		Rodriguez et al. 2002 As(+3)	
91	Mouse (CD)	3 gen (W)				1 (decreased litter size)	Schroeder and Mitchener 1971 As(+3)	
CHRONIC EXPOSURE								
Death								
92	Human	2-7 yr children (W)				0.05 (death)	Zaldivar and Guillier 1977 NS	
93	Human	22 yr (W)				0.014 M (death)	Zaldivar et al. 1981 NS	Cause of death was liver tumor.
94	Monkey (Rhesus)	1 yr (IN)				3 (2/7 died)	Heywood and Sortwell 1979 As(+5)	
95	Rat (Wistar)	27 mo (F)				30 (increased mortality)	Kroes et al. 1974 As(+5) lead arsenate	
96	Mouse (CD)	2 yr (W)				1 (increased mortality, decreased life span)	Schroeder and Balassa 1967 As(+3)	

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
97	Dog (Beagle)	2 yr (F)				2.4 (6/6 died)	Byron et al. 1967 As(+3)	
98	Dog (Beagle)	2 yr (F)				2.4 (1/6 died)	Byron et al. 1967 As(+5)	
Systemic								
99	Human	NS (W)	Resp	0.032	(cough)		Ahmad et al. 1997 NS	
			Dermal			0.032 (melanosis, keratosis, hyperkeratosis, and depigmentation)		
			Ocular			0.032 (chronic conjunctivitis)		
100	Human	>8 yr (W)	Dermal	0.0012	(increased risk of premalignant skin lesions)		Ahsan et al. 2006 (NS)	
101	Human	4 yr (IN)	Dermal			0.1 F (de-pigmentation with hyperkeratosis, possibly pre-cancerous)	Bickley and Papa 1989 As(+3)	
102	Human	NS (W)	Cardio			0.014 (gangrene of feet)	Biswas et al. 1998 NS	
			Dermal			0.014 (melanosis and keratosis of hand palms and foot soles)		

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form	Comments
103	Human	12 yr (W)	Cardio				0.02	(Raynaud's disease, gangrene of toes)	Borgono and Greiber 1972 NS	
			Gastro		0.02	(diarrhea, abdominal pain)				
			Dermal				0.02	(abnormal pigmentation with hyperkaratosis, possibly pre-cancerous)		
104	Human	11-15 yr (W)	Dermal		0.01	(hypo- and hyperpigmentation)			Borgono et al. 1980 NS	
105	Human	NS (W)	Gastro	0.0004	0.022	(gastrointestinal irritation, diarrhea, nausea)			Cebrian et al. 1983 As(+5)	
			Dermal	0.0004			0.022	(pigmentation changes with hyperkeratosis, possibly pre-cancerous)		
106	Human	1-11 yr (W)	Hepatic		0.046	(hepatomegaly)			Chakraborty and Saha 1987 NS	
			Dermal				0.046	(pigmentation changes with keratosis, possibly pre-cancerous)		

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form	Comments
107	Human	NS (W)	Cardio			0.064	(Blackfoot disease)	Chen et al. 1988b NS	
108	Human	>10 yr (W)	Cardio	0.0008		0.022	(increased risk of ischemic heart disease mortality)	Chen et al. 1996 NS	
109	Human	NS (W)	Cardio			0.002	(increased prevalence of cerebrovascular disease and cerebral infarction)	Chiou et al. 1997 NS	
110	Human	>5 yr (W)	Hemato	0.006[c] M 0.007 F				EPA 1981b NS	
			Dermal	0.0009[c] M 0.001 F					
111	Human	3-7 yr (W)	Cardio			0.05	(Blackfoot disease)	Foy et al. 1992 NS	
			Dermal			0.05	(melanosis with hyperkeratosis, possibly pre-cancerous)		

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
112	Human	2-6 yr (IN)	Hepatic			0.08 M (cirrhosis, ascites)	Franklin et al. 1950 As(+3)	
			Dermal			0.08 M (pigmentation with hyperkeratosis, possibly pre-cancerous)		
113	Human	1-15 yr (W)	Hepatic		0.16 (portal fibrosis of the liver)		Guha Mazumder 2005 (NS)	
114	Human	NS (W)	Hepatic	0.004		0.014 (hepatomegaly)	Guha Mazumder et al. 1988 NS	
			Dermal	0.004		0.014 (pigmentation changes with hyperkaratosis, possibly pre-cancerous)		
115	Human	1-20 yr (W)	Gastro		0.06 (abdominal pain)		Guha Mazumder et al. 1988 NS	
			Hemato		0.06 (anemia)			
			Hepatic			0.06 (hepatomegaly, fibrosis)		
			Dermal			0.06 (hyperpigmentation with hyperkeratosis, possibly pre-cancerous)		
116	Human	NS (W)	Dermal	0.0016		0.009 (hyperpigmentation with keratosis, possibly pre-cancerous)	Guha Mazumder et al. 1998a NS	

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
117	Human	(W)	Dermal		0.0014 (arsenical dermatosis)		Guo et al. 2001a (NS)	
118	Human	NS (W)	Dermal		0.0043 (hyperkeratosis, hyperpigmentation)		Haque et al. 2003 (NS)	dose listed is that associated with lowest known peak As concentration ingested by a case with complete water history
119	Human	10 yr (W)	Gastro	0.0046			Harrington et al. 1978 NS	
			Hemato	0.0046				
			Dermal	0.0046				
120	Human	NS (W)	Hepatic	0.0008	0.006 (increased serum alkaline phosphatase and bilirubin)		Hernandez-Zavala et al. 1998 NS	
121	Human	lifetime (W)	Hemato		0.002 F (anemia during pregnancy)		Hopenhayn et al. 2006 (NS)	
122	Human	NS (W)	Cardio			0.067 (ischemic heart disease)	Hsueh et al. 1998b NS	

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
123	Human	0.5-14 yr (W)	Dermal			0.05 (hyperpigmentation with keratosis, possibly pre-cancerous)	Huang et al. 1985 NS	
124	Human	15 yr (IN)	Gastro			0.03 M (hematemesis, hemoperitoneum, melena)	Lander et al. 1975 As(+3)	
			Dermal			0.03 M (hyperkeratosis - possibly pre-cancerous)		
125	Human	NS (W)	Cardio	0.004		0.005 (cyanosis of extremities, palpitations/chest discomfort)	Lianfang and Jianzhong 1994 NS	
			Dermal	0.004		0.005 (keratosis, hyperpigmentation, depigmentation)		
126	Human	3-22 yr (IN)	Gastro			0.05 M (gastrointestinal hemorrhages)	Morris et al. 1974 As(+3)	
			Hepatic			0.05 M (vascular fibrosis, portal hypertension)		
			Dermal			0.05 M (hyperpigmentation with keratoses, possibly pre-cancerous)		

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
127	Human	15 yr (IN)	Hepatic			0.05 F (central fibrosis)	Piontek et al. 1989 As(+3)	
			Dermal			0.05 F (hyperkeratosis, possibly pre-cancerous)		
128	Human	NS (W)	Endocr			0.11 (diabetes mellitus)	Rahman et al. 1998 NS	
129	Human	NS (W)	Cardio	0.018	0.055 (hypertension)		Rahman et al. 1999 NS	

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
130	Human	28 mo (IN)	Cardio	0.06 F			Silver and Wainman 1952 As(+3)	
			Gastro		0.06 F (intermittent, progressively severe nausea, cramps, and diarrhea)			
			Hemato	0.06 F				
			Hepatic		0.06 F (hepatomegaly, fatty liver)			
			Renal	0.06 F				
			Dermal			0.06 F (melanosis with hyperkeratosis, possibly pre-cancerous)		
			Ocular		0.06 F (conjunctival injection, periocular edema)			
131	Human	55 yr (IN)	Hepatic			0.03 M (portal fibrosis and hypertension, bleeding from esophageal varices)	Szuler et al. 1979 As(+3)	
			Dermal			0.03 M (hyperpigmentation with hyperkeratosis, possibly pre-cancerous)		
132	Human	45 yr (W)	Cardio			0.014 (Blackfoot disease)	Tseng 1977 NS	

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
133	Human	NS (W)	Cardio			0.014 (Blackfoot disease)	Tseng 1989 NS	
134	Human	>45 yr (W)	Dermal	0.0008[d] M	0.014 M (hyperkeratosis and hyperpigmentation)		Tseng et al. 1968 NS	
135	Human	>30 yr (W)	Cardio		0.064 M (deficits in cutaneous microcirculation of the toes)		Tseng et al. 1995 As(+3)	
136	Human	52.6 yr (avg) (W)	Cardio	0.016		0.031 (peripheral vascular disease)	Tseng et al. 1996 NS	
137	Human	16 mo (IN)	Resp	0.1 M			Wade and Frazer 1953 As(+3)	
			Cardio	0.1 M				
			Hemato	0.1 M				
			Hepatic		0.1 M (liver enlargment)			
			Dermal			0.1 M (hyperkeratosis, hyperpigmentation with hyperkeratosis, possibly pre-cancerous)		

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
138	Human	30-33 yr (W)	Dermal			0.015 M (hyperkeratosis of foot, possibly pre-cancerous)	Zaldivar 1974 NS	
139	Human	12 yr (W)	Resp		0.015[c] M (bronchitis, bronchiectasis)		Zaldivar 1974 NS	
					0.018 F (bronchitis, bronchiectasis)			
			Cardio			0.015[c] M (Raynaud's disease, thrombosis)		
						0.018 F		
			Gastro		0.015[c] M (diarrhea)			
					0.018 F (diarrhea)			
			Dermal		0.015[c] M (scaling of skin, hyperkeratosis, leukoderma, melanoderma)			
					0.018 F			
			Bd Wt		0.015[c] M (unspecified decreased body weight)			
					0.018 F (unspecified decreased body weight)			

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
140	Human	NS (W)	Dermal			0.063 (hyperpigmentation with keratoses, possibly pre-cancerous)	Zaldivar 1977 NS	
141	Human	2-7 yr children (W)	Resp			0.08 (inflammation of bronchi and larynx, bronchopneumonia)	Zaldivar and Guillier 1977 NS	
			Cardio			0.05 (vascular spasms, thrombosis, ischemia, hypotension, cardiac failure)		
			Gastro			0.05 (nause, vomiting, diarrhea, intestinal hemorrhage)		
			Hemato			0.05 (anemia)		
			Hepatic			0.08 (cirrhosis)		
			Renal		0.08 (cloudy swelling in kidneys)			
			Dermal			0.05 (hyperkeratosis of palms and soles, melanoderma, leukoderma)		
142	Human	1-39 yr (W)	Cardio			0.06 (arterial thickening, Raynaud's disease)	Zaldivar and Guillier 1977 NS	

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
143	Rat (Osborne-Mendel)	2 yr (F)	Resp	20			Byron et al. 1967 As(+3)	
			Cardio	20				
			Gastro	20				
			Hemato	9	20 (slight transient decrease in Hb and Hct values)			
			Hepatic	4		9 (enlarged bile duct, bile duct proliferation)		
			Renal	9	20 (pigmentation)			
			Bd Wt	2	4 (decreased body weight gain)			
144	Rat (Osborne-Mendel)	2 yr (F)	Resp	30			Byron et al. 1967 As(+5)	
			Cardio	30				
			Gastro	30				
			Hemato	30				
			Hepatic	9	20 (enlarged bile duct)			
			Renal	9	20 (pigmentation, cysts)			
			Bd Wt		2 (decreased body weight gain in females)			

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
145	Rat (Wistar)	27 mo (F)	Resp	7			Kroes et al. 1974 As(+5)	
			Cardio	7				
			Gastro	7				
			Hemato	7				
			Musc/skel	7				
			Hepatic	7				
			Renal	7				
			Endocr	7				
			Bd Wt		7 (decreased body weight gain)			

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
146	Rat (Wistar)	27 mo (F)	Resp	30			Kroes et al. 1974 As(+5) lead arsenate	
			Cardio	30				
			Gastro	30				
			Hemato	7	30 (slight anemia)			
			Musc/skel	30				
			Hepatic	7		30 (enlarged bile duct with extensive dilation and inflammation)		
			Renal	30				
			Endocr	30				
			Bd Wt	7	30 (decreased body weight gain)			
147	Rat (Long-Evans)	3 yr (W)	Resp	0.6			Schroeder et al. 1968 As(+3)	
			Cardio	0.6				
			Hepatic	0.6				
			Renal	0.6				
			Dermal	0.6				
			Bd Wt	0.6				

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
148	Mouse (NS)	48 wk (W)	Hepatic	11.1			Liu et al. 2000 As(+3)	
			Renal		5.6 (histological alterations of the kidney)			
			Bd Wt	11.1				
149	Mouse (NS)	48 wk (W)	Hepatic	18.5			Liu et al. 2000 As (+5)	
			Renal		18.5 (increased relative kidney weight)			
			Bd Wt	18.5				
150	Mouse (BALB/c)	15 mo (W)	Hepatic		0.7 M (increased liver weight, altered liver histopathology, decreased hepatic enzymes in serum)		Santra et al. 2000 (NS)	
			Bd Wt		0.7 M (13-17% decreased body weight)			
151	Mouse (CD)	2 yr (W)	Bd Wt		1 (decreased body weight gain after the first 6 months of the study)		Schroeder and Balassa 1967 As(+3)	

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
152	Dog (Beagle)	2 yr (F)	Resp	2.4			Byron et al. 1967 As(+3)	
			Cardio	2.4				
			Gastro	1		2.4 (bleeding in the gut)		
			Hemato	1	2.4 (slight to moderate anemia)			
			Hepatic	1	2.4 (hemosiderin deposits in hepatic macrophages)			
			Renal	2.4				
			Bd Wt	1		2.4 (44-61% weight loss)		
153	Dog (Beagle)	2 yr (F)	Resp	2.4			Byron et al. 1967 As(+5)	
			Cardio	2.4				
			Gastro	2.4				
			Hemato	1	2.4 (mild anemia)			
			Hepatic	1	2.4 (pigmentation in hepatic macrophages)			
			Renal	2.4				
			Bd Wt	1		2.4 (marked decreased weight gain)		

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Neurological								
154	Human	>5 yr (W)		0.006[c] M 0.007 F			EPA 1981b NS	
155	Human	3-7 yr (W)				0.11 F (wrist weakness)	Foy et al. 1992 NS	
156	Human	1-20 yr (W)			0.06 (tingling of hands and feet)		Guha Mazumder et al. 1988 NS	
157	Human	10 yr (W)		0.0046			Harrington et al. 1978 NS	
158	Human	NS (W)		0.0014		0.04 (functional denervation)	Hindmarsh et al. 1977 NS	
159	Human	NS (W)		0.004	0.005 (fatigue, headache, dizziness, insomnia, nighmare, numbness)		Lianfang and Jianzhong 1994 NS	
160	Human	28 mo (IN)				0.06 F (paresthesia)	Silver and Wainman 1952 As(+3)	
161	Human	55 yr (IN)			0.03 M (absent ankle jerk reflex and vibration sense in legs)		Szuler et al. 1979 As(+3)	

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
162	Human	NS (W)			0.0017 (decreased performance in neurobehavioral tests)		Tsai et al. 2003 (NS)	
163	Human	lifetime continuous (W)			0.005 (decreased performance in neurobehavioral tests)		Wasserman et al. 2004 (NS)	
164	Human	lifetime		0.0008	0.003 (decreased score in Performance domain of an intelligence scale)		Wasserman et al. 2007 (NS)	
Reproductive								
165	Human	NS (W)				0.008 F (increased frequencies for spontaneous abortion, stillbirth, and preterm birth rates)	Ahmad et al. 2001 (NS)	98% of the exposed group drank water containing 0.1 mg As/L or more.
166	Human	lifetime (W)				0.006 F (increased incidence of spontaneous abortion)	Milton et al. 2005 (NS)	
167	Human	lifetime (W)				0.02 F (increased risk of stilbirth)	von Ehrenstein et al. 2006 (NS)	

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form	Comments
Developmental										
168	Human	continuous (W)			0.002	(reduced birth weight)			Hopenhayn et al. 2003a (NS)	
169	Human	>1 yr 1 x/d (W)					0.03	(increased SMR for malignant and non-malignant lung disease)	Smith et al. 2006 (NS)	
170	Human	lifetime (W)		0.008					von Ehrenstein et al. 2006 (NS)	NOAEL is for no increase in risk of neonatal mortality or overall infant mortality.
Cancer										
171	Human	NS (W)					0.022	(CEL: skin cancer)	Cebrian et al. 1983 As(+5)	
172	Human	NS (W)					0.064	(CEL: bladder, lung and liver cancers)	Chen et al. 1986 NS	
173	Human	NS (W)					0.064	(CEL: malignant neoplasms of the bladder, skin, lung and liver)	Chen et al. 1988b NS	
174	Human	NS (W)					0.003	(CEL: bladder cancer)	Chiou et al. 2001 (NS)	

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form	Comments
175	Human	2 wk- 12 yr (IN)				3.67	(CEL: bladder cancer risk)	Cuzick et al.1992 As(+3)	
176	Human	NS (W)				0.0011	(CEL: lung cancer)	Ferreccio et al. 1998 NS	
177	Human	NS (W)				0.0017	(CEL: lung cancers)	Ferreccio et al. 2000 (NS)	
178	Human	NS (W)				0.018	(CEL: lung cancer mortality)	Guo 2004 (NS)	
179	Human	NS (W)				0.018	(CEL: bladder cancer)	Guo and Tseng 2000 (NS)	
180	Human	NS (W)				0.052	(CEL: increased incidence of transitional cell carcinomas of the bladder, kidney, ureters, and all urethral cancer)	Guo et al. 1997 NS	
181	Human	NS (W)				0.0049[c] M	(CEL: squamous cell carcinoma of the skin)	Guo et al. 2001b (NS)	
						0.0094 F			

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form	Comments
182	Human	>1 yr (W)				0.0075	(CEL: basal or squamous skin carcinoma)	Haupert et al. 1996 NS	
183	Human	16 yr (avg) (IN)				0.04 M	(CEL: basal cell and squamous cell carcinomas of the skin, small cell and squamous cell carcinoma of the lung)	Luchtrath 1983 As(+5)	
184	Human	60 yr (W)				0.038	(CEL: intraepidermal carcinoma)	Tseng 1977 NS	
185	Human	>45 yr (W)				0.014	(CEL: squamous cell carcinoma of the skin)	Tseng et al. 1968 NS	
186	Human	~5 yr (W)				0.033	(CEL: lung, urinary tract cancer)	Tsuda et al. 1995a As(+3)	

Table 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
187	Human	12 yr (W)				0.015[c] M (CEL: squamous cell carcinoma of the skin)	Zaldivar 1974 NS	
						0.018 F (CEL: squamous cell carcinoma of the skin)		
188	Human	22-34 yr (W)				0.014 M (CEL: basal cell and squamous cell carcinomas of the skin, hemangioendothelioma of the liver)	Zaldivar et al. 1981 NS	

a The number corresponds to entries in Figure 3-3.

b Used to derive provisional acute oral minimal risk level (MRL) of 0.005 mg/kg/day; dose divided by an uncertainty factor of 10 (for extrapolation from a LOAEL to a NOAEL).

c Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 3-3. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

d Used to derive chronic oral minimal risk level (MRL) of 0.0003 mg/kg/day; dose divided by an uncertainty factor of 3 (for human variability).

avg = average; ALAD = delta-aminolevulinic acid dehydratase; ALAS = delta-aminolevulinic acid synthetase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; Bd Wt = body weight; BUN = blood urea nitrogen; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); Endocr = endocrine; (F) = feed; F = female; (G) = gavage; Gastro = gastrointestinal; Gd = gestational day; GI = gastrointestinal; (GW) = gavage in water; gen = generation; Gd = gestation day; Gn pig = guinea pig; Hemato = hematological; Hb = hemoglobin; Hct = hematocrit; Hemato = hematological; hr = hour(s); (IN) = ingestion; LD50 = lethal dose, 50% kill; LDH = lactate dehydrogenase; LOAEL = lowest-observable-adverse-effect level; M = male; Metab = metabolic; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observable-adverse-effect level; NS = not specified; pmd = pre-mating day; pnd = post-natal day; Resp = respiratory; SMR =standardized mortality ratio; (W) = drinking water; wk = week(s); x = time(s); yr = year(s)

Figure 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral

Acute (≤14 days)

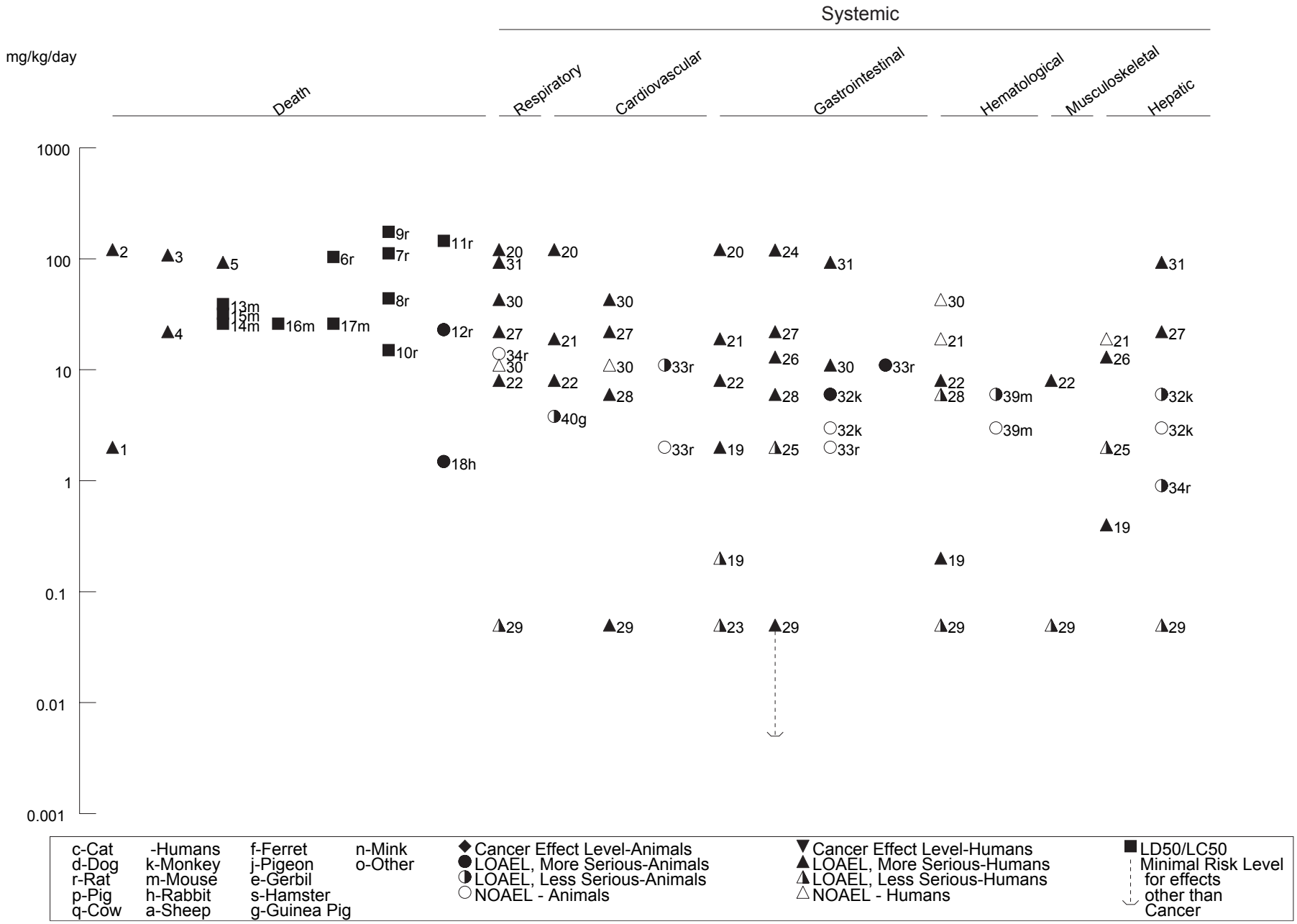
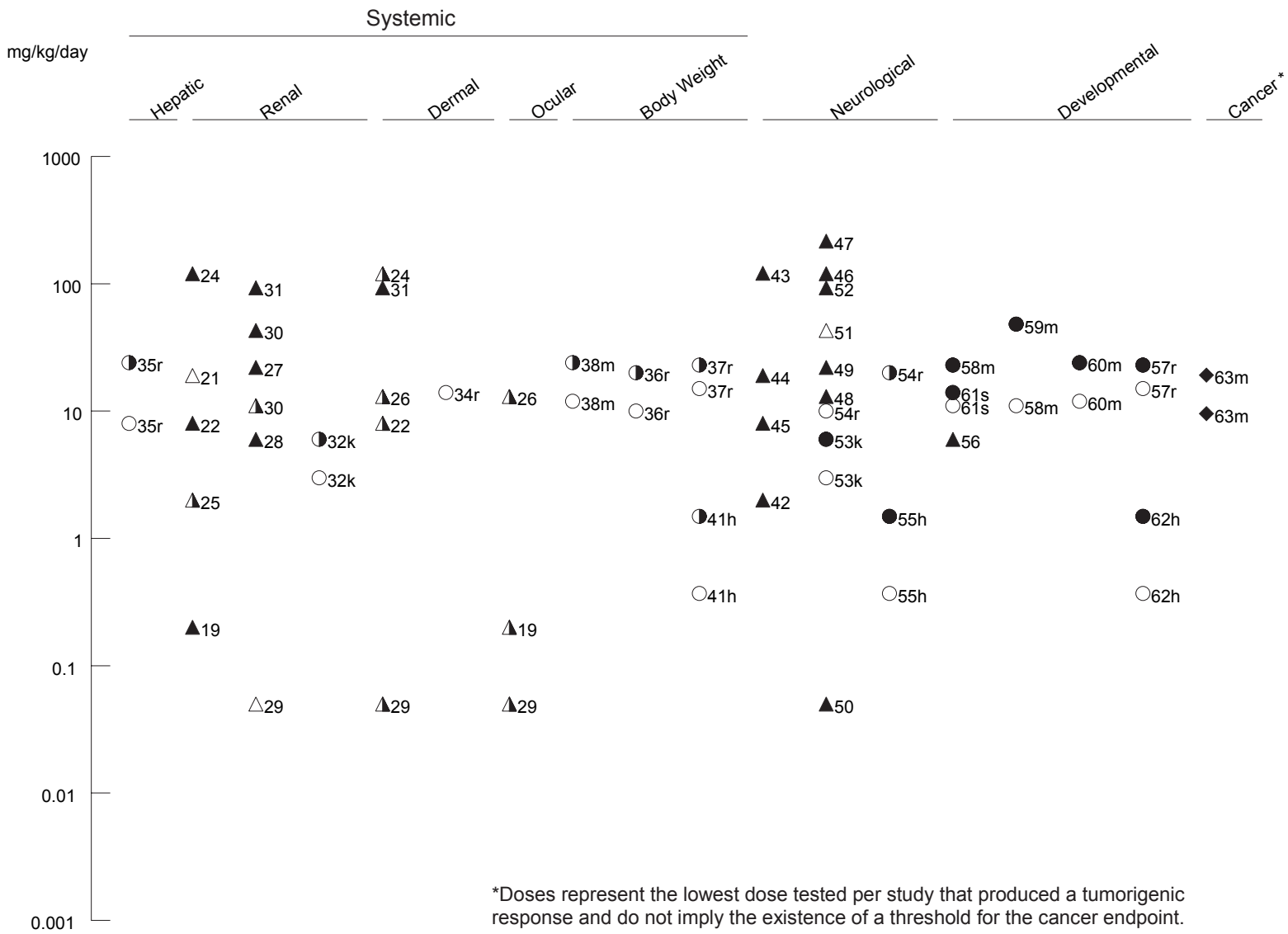


Figure 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral *(Continued)*

Acute (≤14 days)

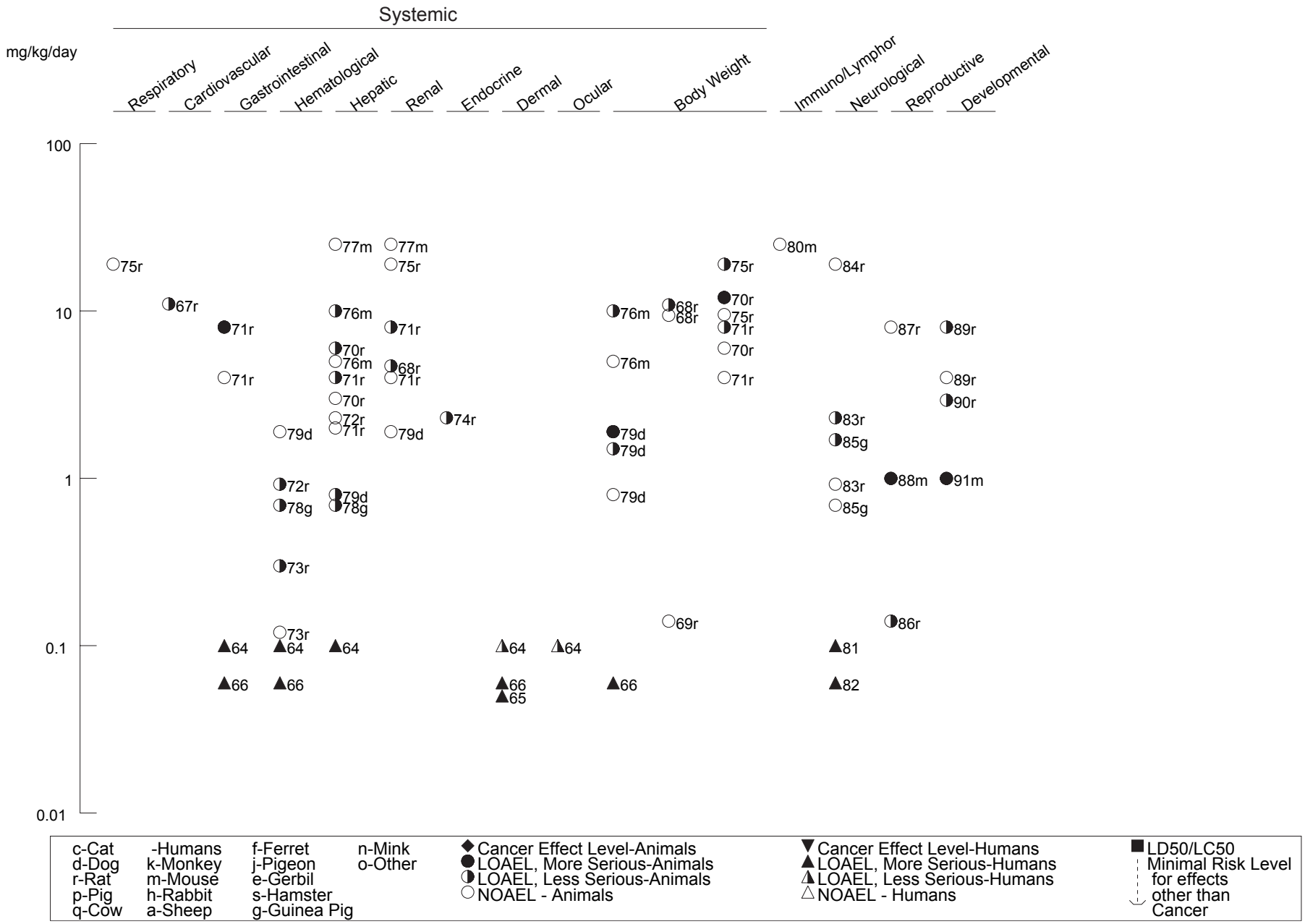


*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint.

c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level for effects other than Cancer
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◭ LOAEL, Less Serious-Humans	
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	
q-Cow	a-Sheep	g-Guinea Pig				

Figure 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral *(Continued)*

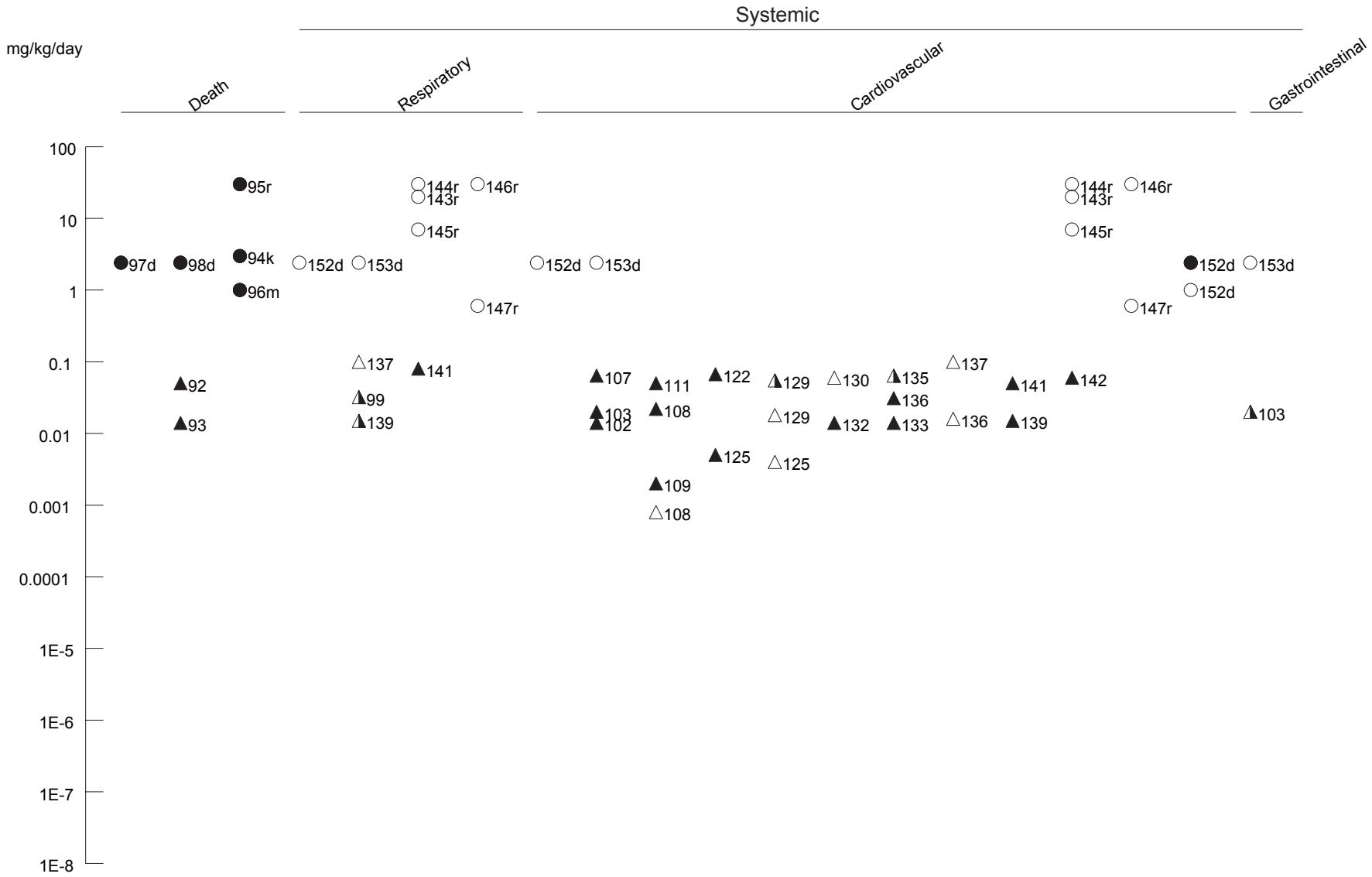
Intermediate (15-364 days)



c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level for effects other than Cancer
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◮ LOAEL, Less Serious-Humans	
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	
q-Cow	a-Sheep	g-Guinea Pig				

Figure 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral *(Continued)*

Chronic (≥365 days)



c-Cat	-Humans	f-Ferret	n-Mink
d-Dog	k-Monkey	j-Pigeon	o-Other
r-Rat	m-Mouse	e-Gerbil	
p-Pig	h-Rabbit	s-Hamster	
q-Cow	a-Sheep	g-Guinea Pig	

◆ Cancer Effect Level-Animals
● LOAEL, More Serious-Animals
◑ LOAEL, Less Serious-Animals
○ NOAEL - Animals

▼ Cancer Effect Level-Humans
▲ LOAEL, More Serious-Humans
◮ LOAEL, Less Serious-Humans
△ NOAEL - Humans

■ LD50/LC50
Minimal Risk Level for effects other than Cancer

Figure 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral *(Continued)*

Chronic (≥365 days)

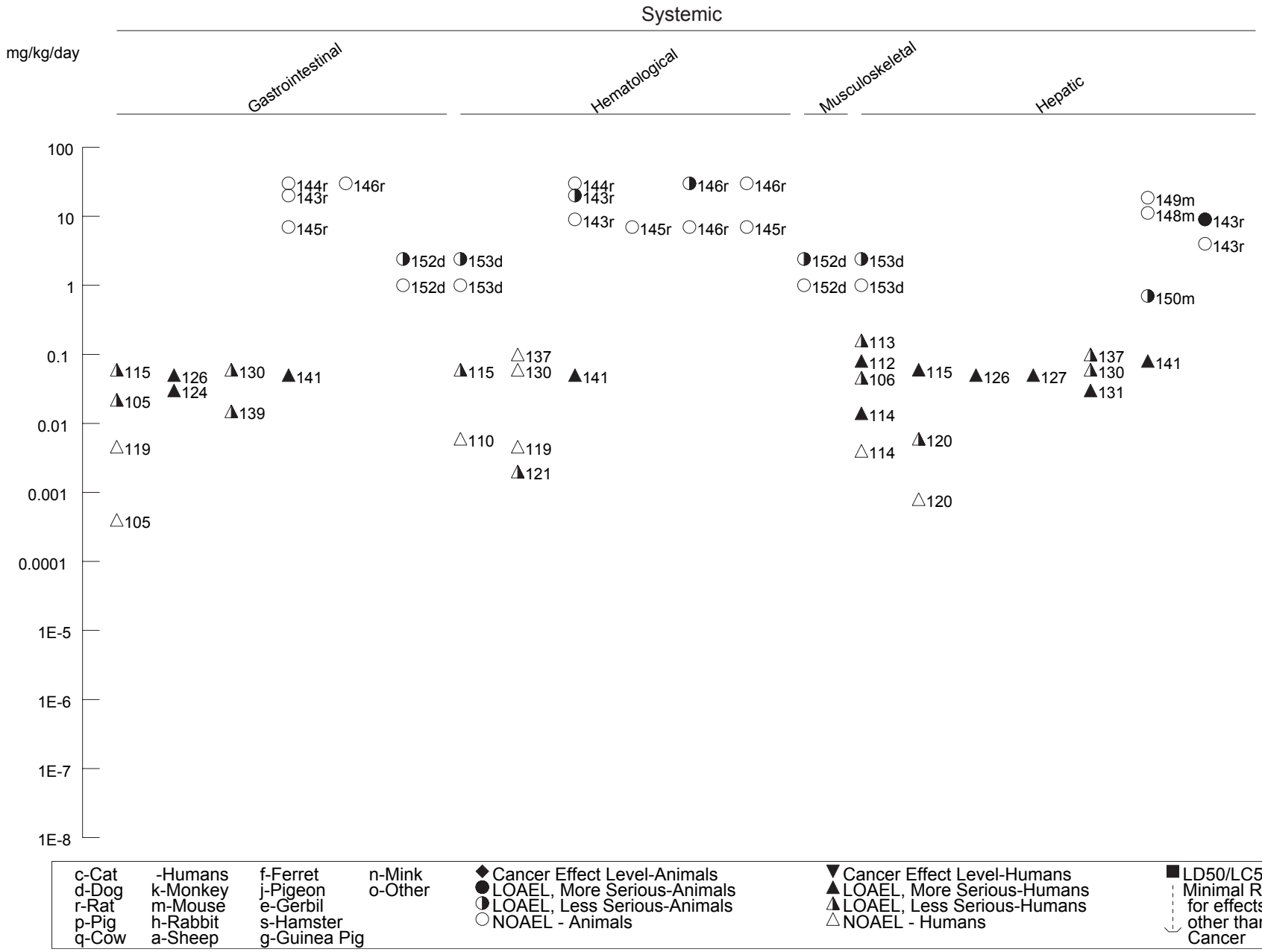
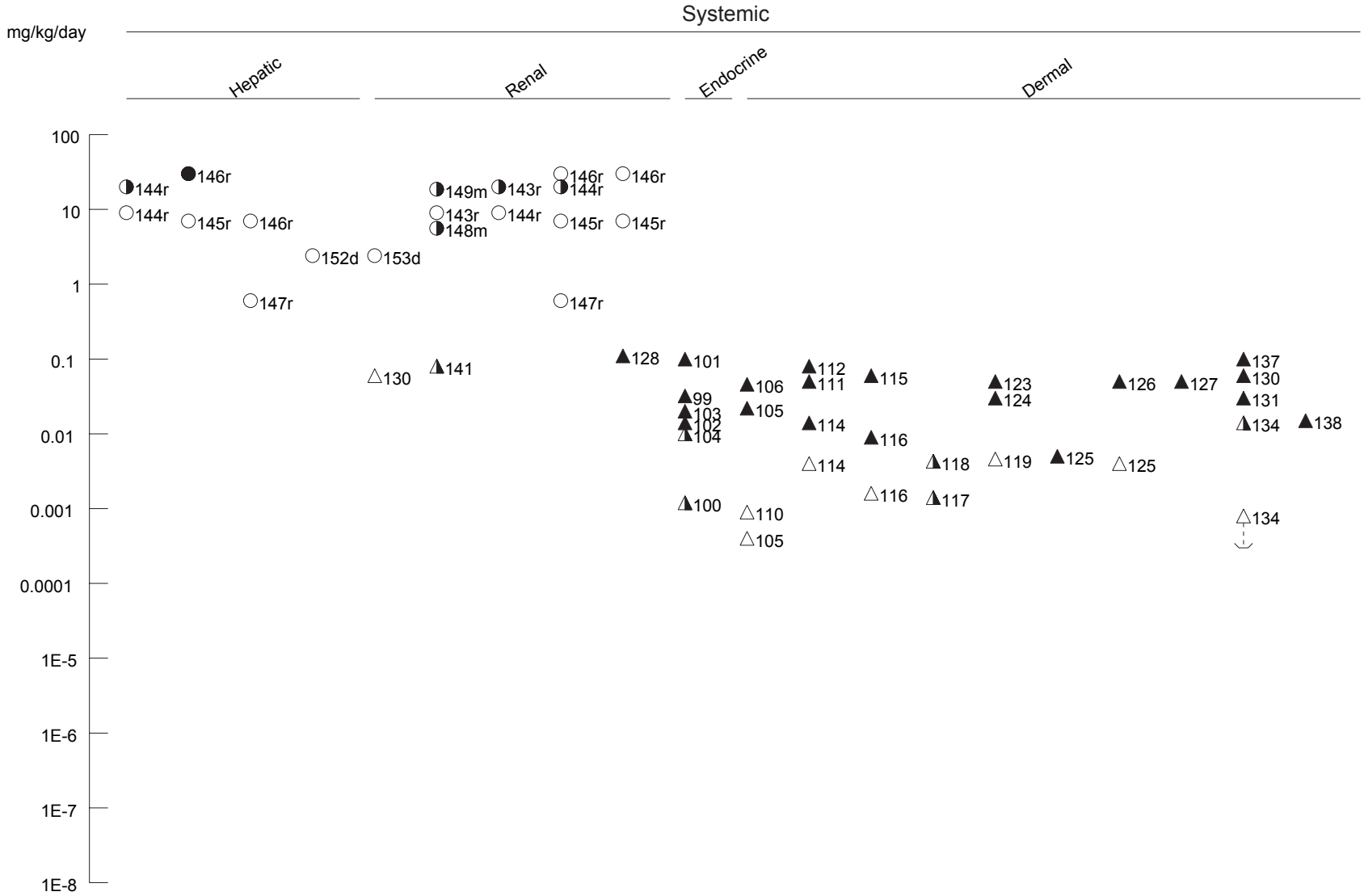


Figure 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral *(Continued)*

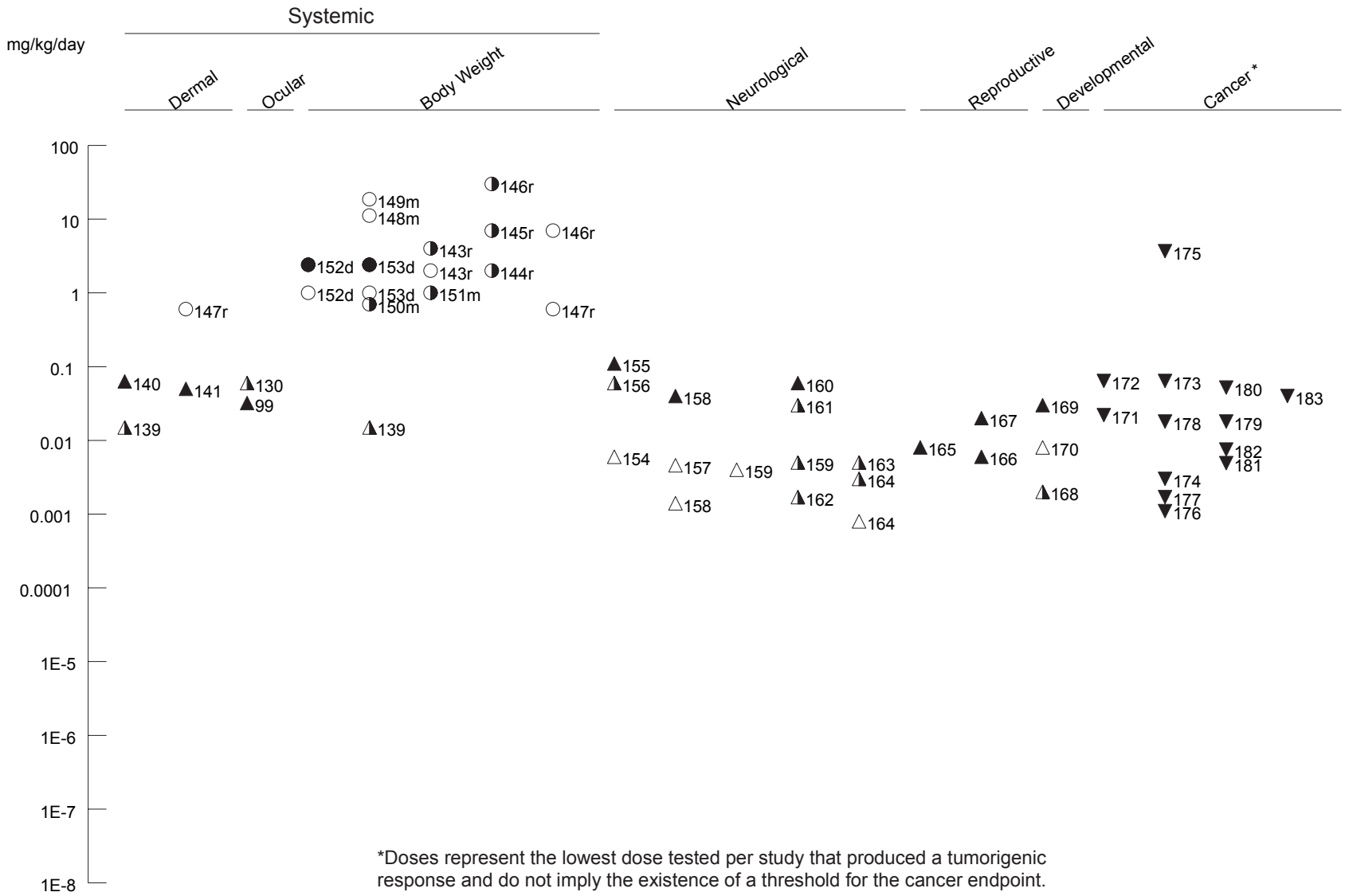
Chronic (≥365 days)



c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level for effects other than Cancer
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◭ LOAEL, Less Serious-Humans	
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	
q-Cow	a-Sheep	g-Guinea Pig				

Figure 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral *(Continued)*

Chronic (≥365 days)

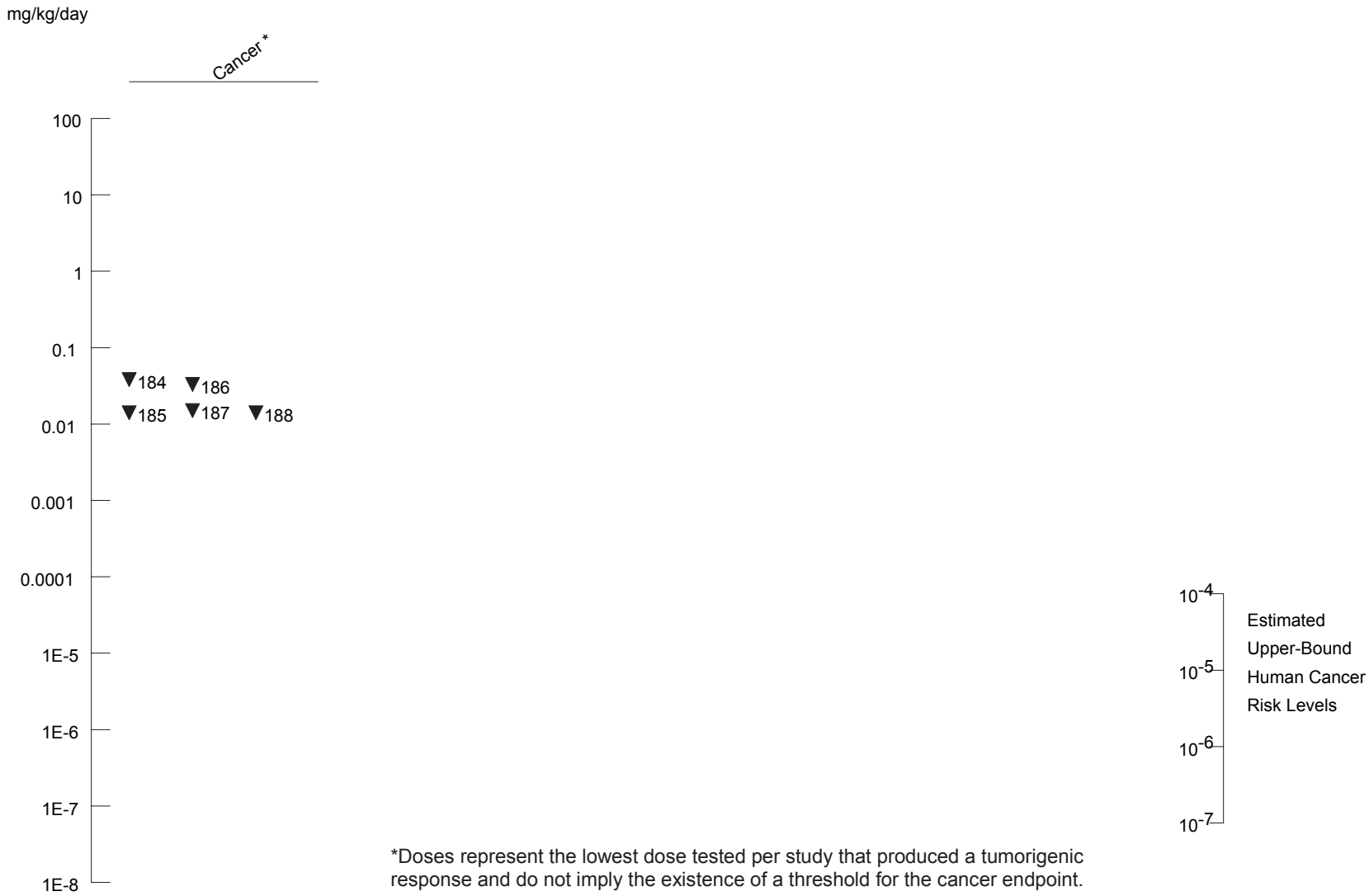


*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint.

c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level for effects other than Cancer
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◭ LOAEL, Less Serious-Humans	
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	
q-Cow	a-Sheep	g-Guinea Pig				

Figure 3-3 Levels of Significant Exposure to Inorganic Arsenic - Oral *(Continued)*

Chronic (≥365 days)



*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint.

c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level for effects other than Cancer
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◭ LOAEL, Less Serious-Humans	
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	
q-Cow	a-Sheep	g-Guinea Pig				

Table 3-4 Levels of Significant Exposure to Monomethylarsonic Acid - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Rat (Sprague-Dawley)	once (GW)				3184 M (LD50) 2449[b] F (LD50)	Gur and Nyska 1990 MSMA	
2	Mouse (ddY)	once (GW)				1800 M (LD50)	Kaise et al. 1989 MMA	
3	Rabbit (New Zealand)	once (GW)				102 M (LD50)	Jaghabir et al. 1988 MSMA	
Systemic								
4	Rat (Sprague-Dawley)	once (GW)	Gastro		2030 (mucoid feces and diarrhea)		Gur and Nyska 1990 MSMA	
			Bd Wt	2030				
5	Rat (Sprague-Dawley)	Gd 6-15 (GW)	Bd Wt	10 F	100 F (17% decrease in maternal body weight gain)	500 F (40% decrease in maternal body weight gain)	Irvine et al. 2006 MMA	
6	Mouse (ddY)	once (GW)	Resp			1800 M (respiratory arrest)	Kaise et al. 1989 MMA	
			Gastro		2200 M (diarrhea, slight congestion of the small intestine)			

Table 3-4 Levels of Significant Exposure to Monomethylarsonic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
7	Rabbit (New Zealand)	Gd 7-19 (GW)	Gastro	7 F	12 F (loose feces/diarrhea in 7/14 pregnant rabbits)		Irvine et al. 2006 MMA	
			Bd Wt	7 F		12 F (67% decrease in maternal body weight gain)		
8	Rabbit (New Zealand)	once (GW)	Gastro		60 M (diarrhea)		Jaghabir et al. 1988 MSMA	
Developmental								
9	Rat (Sprague-Dawley)	Gd 6-15 (GW)		100	500 (decreased fetal weight and increased fetal incidence of imcomplete ossification of thoracic vertebrae)		Irvine et al. 2006 MMA	
10	Rabbit (New Zealand)	Gd 7-19 (GW)		7	12 (supernumerary thoracic ribs and eight lumbar vertebrae)		Irvine et al. 2006 MMA	
INTERMEDIATE EXPOSURE								
Death								
11	Rat (Fischer- 344)	52 wk (F)				106.9 M (increased mortality)	Arnold et al. 2003 MMA	

Table 3-4 Levels of Significant Exposure to Monomethylarsonic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Systemic								
12	Rat (Fischer- 344)	52 wk (F)	Gastro	3.5[c] M	30.2 M (diarrhea)		Arnold et al. 2003 MMA	
			Bd Wt	30.2 M	106.9 M (14% decrease in body weight)			
13	Rat (Sprague-Dawley)	146-171 d pre-mating, mating, gestation, and lactation (F)	Bd Wt	76			Schroeder 1994 MMA	
14	Dog (Beagle)	52 wk (C)	Gastro		2 M (diarrhea)		Waner and Nyska 1988 MMA	
			Bd Wt	2 F	8 F (decrease in body weight)			
Reproductive								
15	Rat (Sprague-Dawley)	146-171 d pre-mating, mating, gestation, and lactation (F)		22	76 (decreased pregnancy rate and male fertility index in F0 and F1)		Schroeder 1994 MMA	
16	Mouse (Swiss)	19 d 3 d/wk (GW)			119 M (reduced fertility)		Prukop and Savage 1986 MSMA	

Table 3-4 Levels of Significant Exposure to Monomethylarsonic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Developmental								
17	Rat (Sprague-Dawley)	146-171 d pre-mating, mating, gestation, and lactation (F)		22	76 (decreased pup survival F1 and F2)		Schroeder 1994 MMA	
CHRONIC EXPOSURE								
Death								
18	Rat (Fischer- 344)	104 wk (F)				72.4 M (increased mortality)	Arnold et al. 2003 MMA	

Table 3-4 Levels of Significant Exposure to Monomethylarsonic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Systemic								
19	Rat (Fischer- 344)	104 wk (F)	Gastro	3 M	25.7 M (diarrhea)	72.4 M (necrosis, ulceration, perforation in large intestine)	Arnold et al. 2003 MMA	
			Hemato	72.4 M				
			Musc/skel	72.4 M				
			Hepatic	72.4 M				
			Renal	3.9 F	33.9 F (increased absolute kidney weight and progressive glomerulonephropathy)			
			Endocr	3.9 F	33.9 F (hypertrophy of thyroid follicular epithelium, decreased absolute thyroid weight)			
			Dermal	72.4 M				
			Ocular	72.4 M				
			Bd Wt	3 M	25.7 M (15% decrease in body weight)	33.9 F (30% decrease in body weight)		

Table 3-4 Levels of Significant Exposure to Monomethylarsonic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
20	Rat (Fischer- 344)	104 wk (W)	Hemato	8.4 M			Shen et al. 2003 MMA	
			Hepatic	2.1 M	8.4 M (increased GST-P-positive foci)			
			Renal		2.1 M (hyperplasia of the bladder)			
			Bd Wt	8.4 M				
21	Mouse (B6C3F1)	104 wk (F)	Cardio	67.1 M			Arnold et al. 2003 MMA	
			Gastro	24.9 M	67.1 M (loose and mucoid feces, metaplasia of the cecum and colon)			
			Musc/skel	67.1 M				
			Hepatic	67.1 M				
			Renal	1.2[d] M	6 M (increased incidence of progressive glomeruloephropathy)			
			Dermal	67.1 M				
			Ocular	67.1 M				
			Bd Wt	24.9 M	67.1 M (17% decrease in body weight)			

Table 3-4 Levels of Significant Exposure to Monomethylarsonic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
22	Dog (Beagle)	52 wk (C)	Resp	35			Waner and Nyska 1988 MMA	
			Gastro		2 M (diarrhea)			
			Hemato	35				
			Hepatic	35				
			Renal		8 (increased urine specific gravity; increased kidney weight)			
			Ocular	35				
			Bd Wt	2 F		8 F (42% decrease in body weight)		
Neurological								
23	Dog (Beagle)	52 wk (C)		35			Waner and Nyska 1988 MMA	
Reproductive								
24	Dog (Beagle)	52 wk (C)		35 M 8 F	35 F (decrease in estrus)		Waner and Nyska 1988 MMA	Histological examination of reproductive tissues.

Table 3-4 Levels of Significant Exposure to Monomethylarsonic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Cancer								
25	Rat (Fischer- 344)	104 wk (W)		8.4 M			Shen et al. 2003 MMA	

a The number corresponds to entries in Figure 3-4.

b Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 3-4. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

c The intermediate-duration oral MRL of 0.1 mg MMA/kg/day was calculated using a benchmark dose analysis. The BMDL10 of 12.38 mg MMA/kg/day was divided by an uncertainty factor of 100 (10 to account for extrapolation from animals to humans and 10 for human variability)

d The chronic-duration oral MRL of 0.01 mg MMA/kg/day was calculated using a benchmark dose analysis. The BMDL10 of 1.09 mg MMA/kg/day was divided by an uncertainty factor of 100 (10 to account for extrapolation from animals to humans and 10 for human variability)

Bd Wt = body weight; (C) = capsule; Cardio = cardiovascular; d = day(s); Endocr = endocrine; (F) = feed; F = female; Gastro = gastrointestinal; (GO) = gavage in oil; (GW) = gavage in water; Gd = gestation day; GST-P = glutathione S-transferase placental form; Hemato = hematological; IN = ingestion; LD50 = lethal dose, 50% kill; LOAEL = lowest-observable-adverse-effect level; M = male; MMA = monomethylarsonic acid; MSMA = monosodium methane arsonate; Musc/skel = musculoskeletal; NOAEL = no-observable-adverse-effect level; Resp = respiratory; (W) = drinking water; wk = week(s); x = time(s)

Figure 3-4 Levels of Significant Exposure to Monomethylarsonic Acid - Oral

Acute (≤14 days)

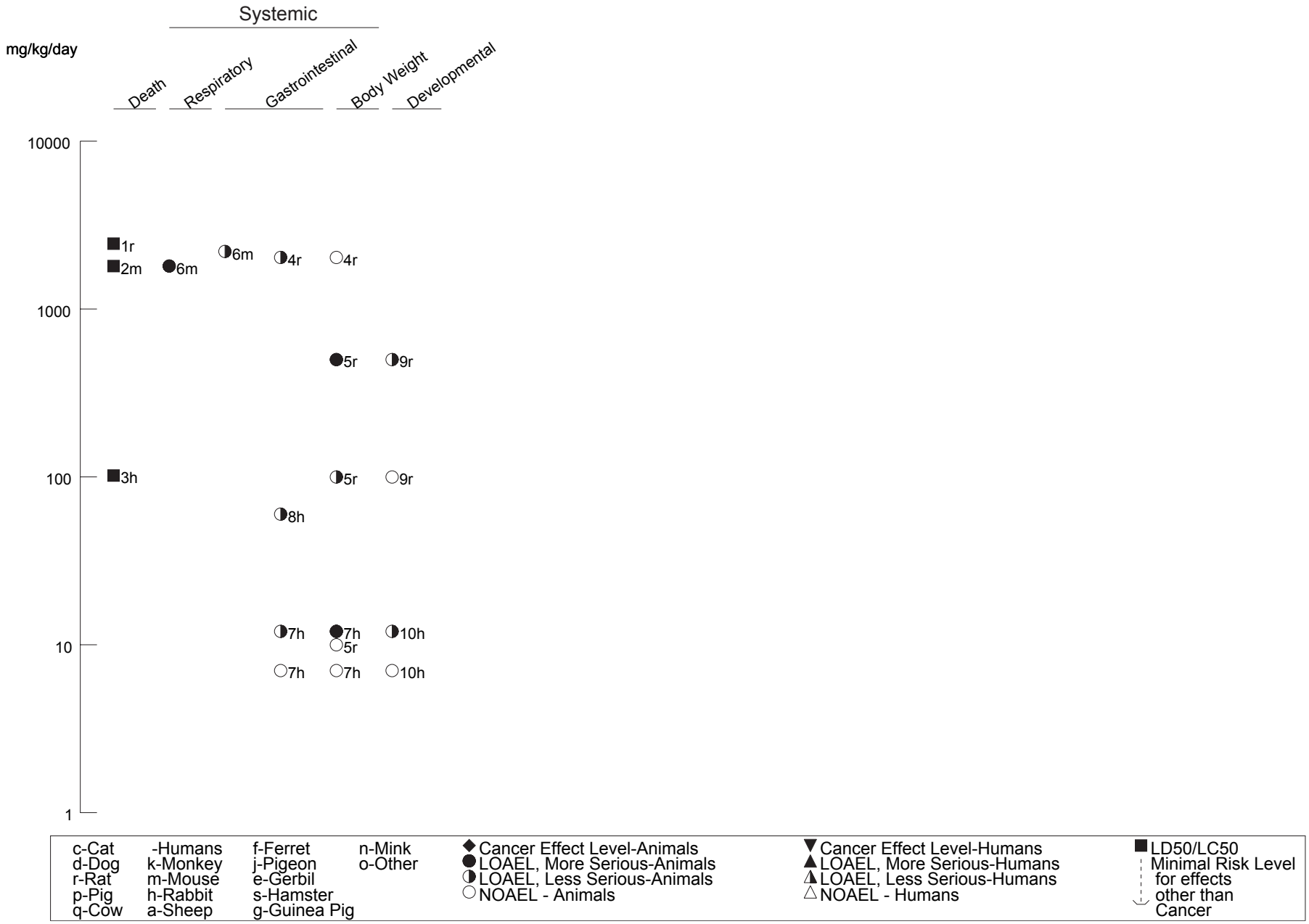


Figure 3-4 Levels of Significant Exposure to Monomethylarsonic Acid - Oral *(Continued)*

Intermediate (15-364 days)

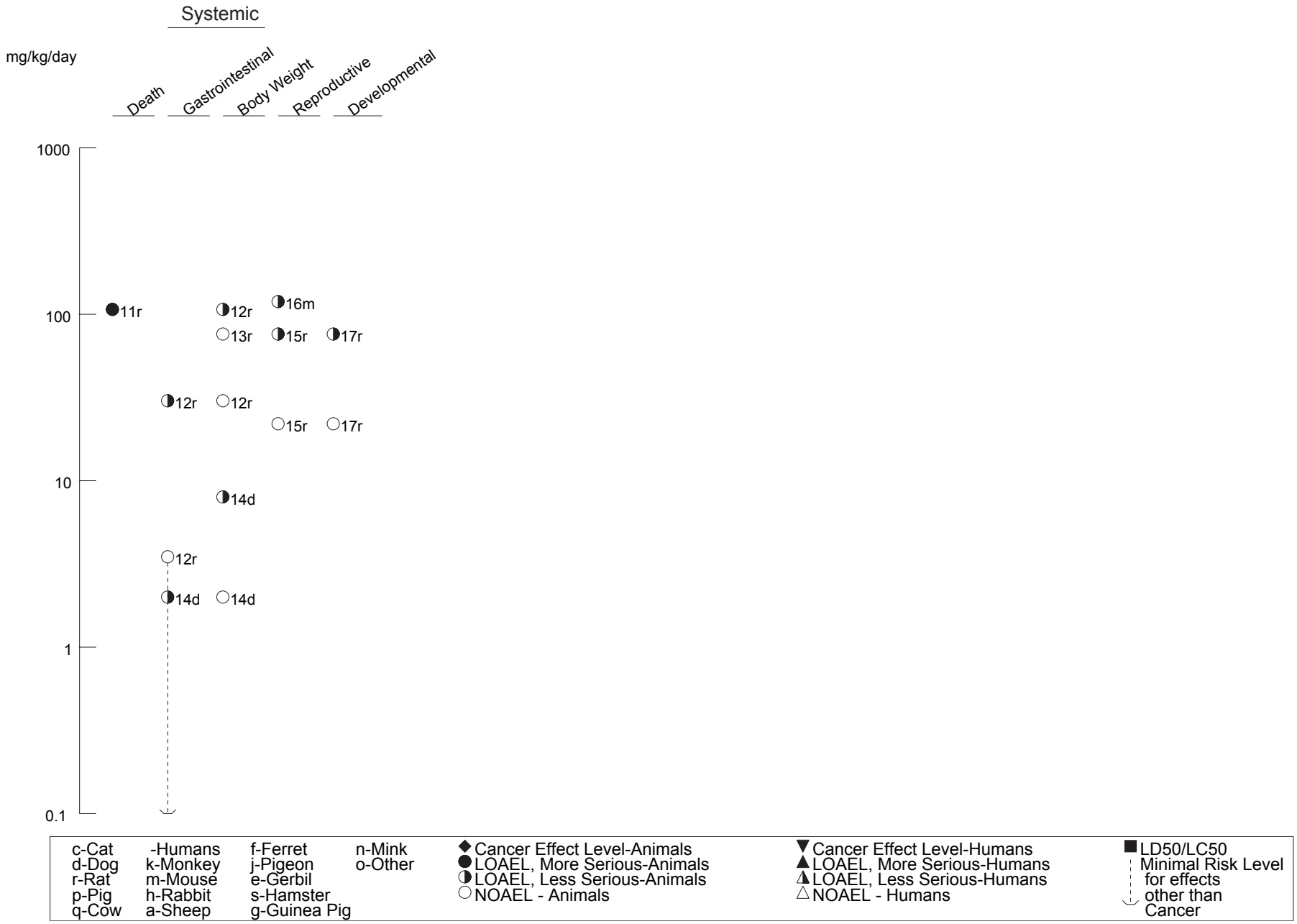


Figure 3-4 Levels of Significant Exposure to Monomethylarsonic Acid - Oral *(Continued)*

Chronic (≥365 days)

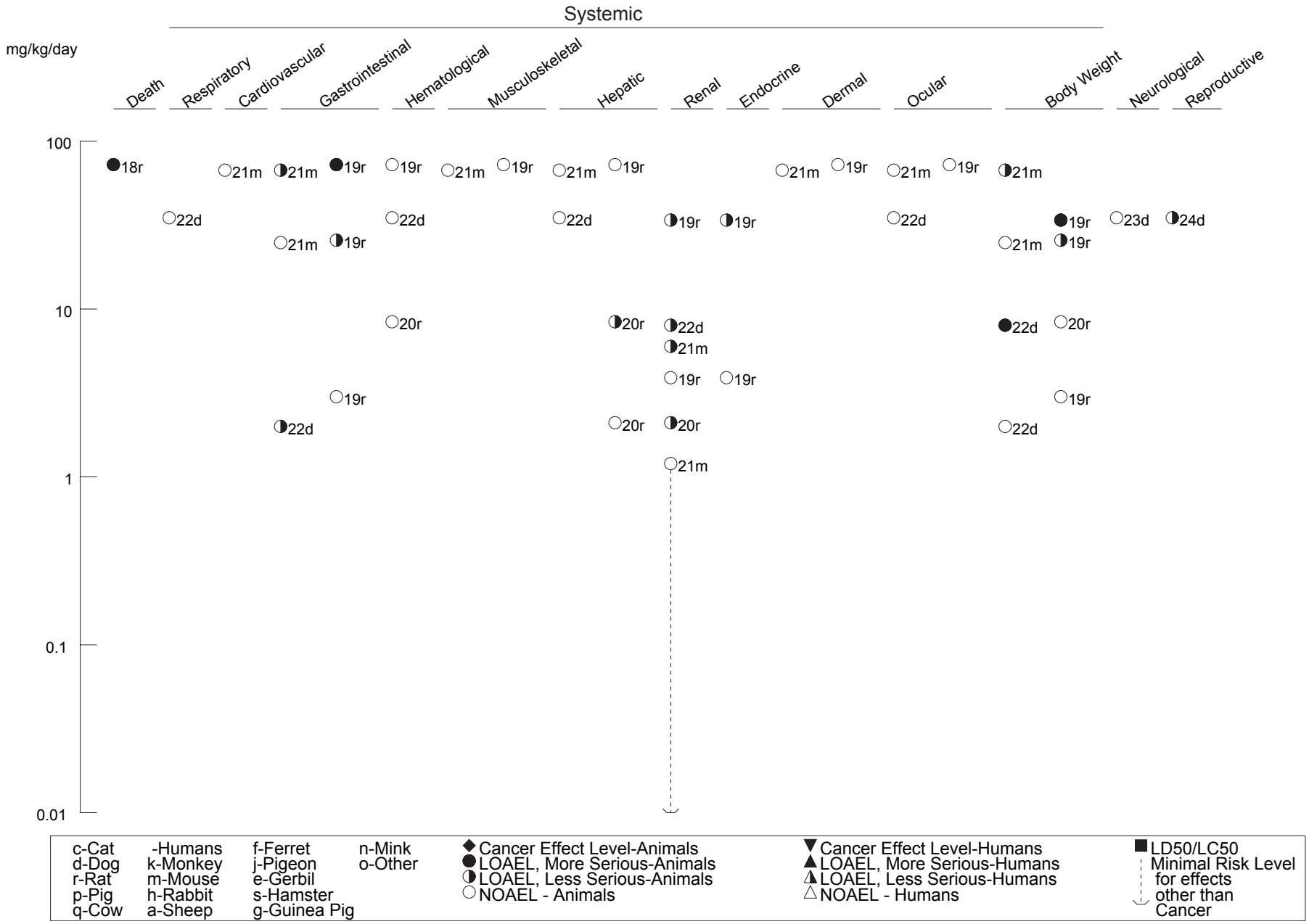


Figure 3-4 Levels of Significant Exposure to Monomethylarsonic Acid - Oral *(Continued)*

Chronic (≥365 days)

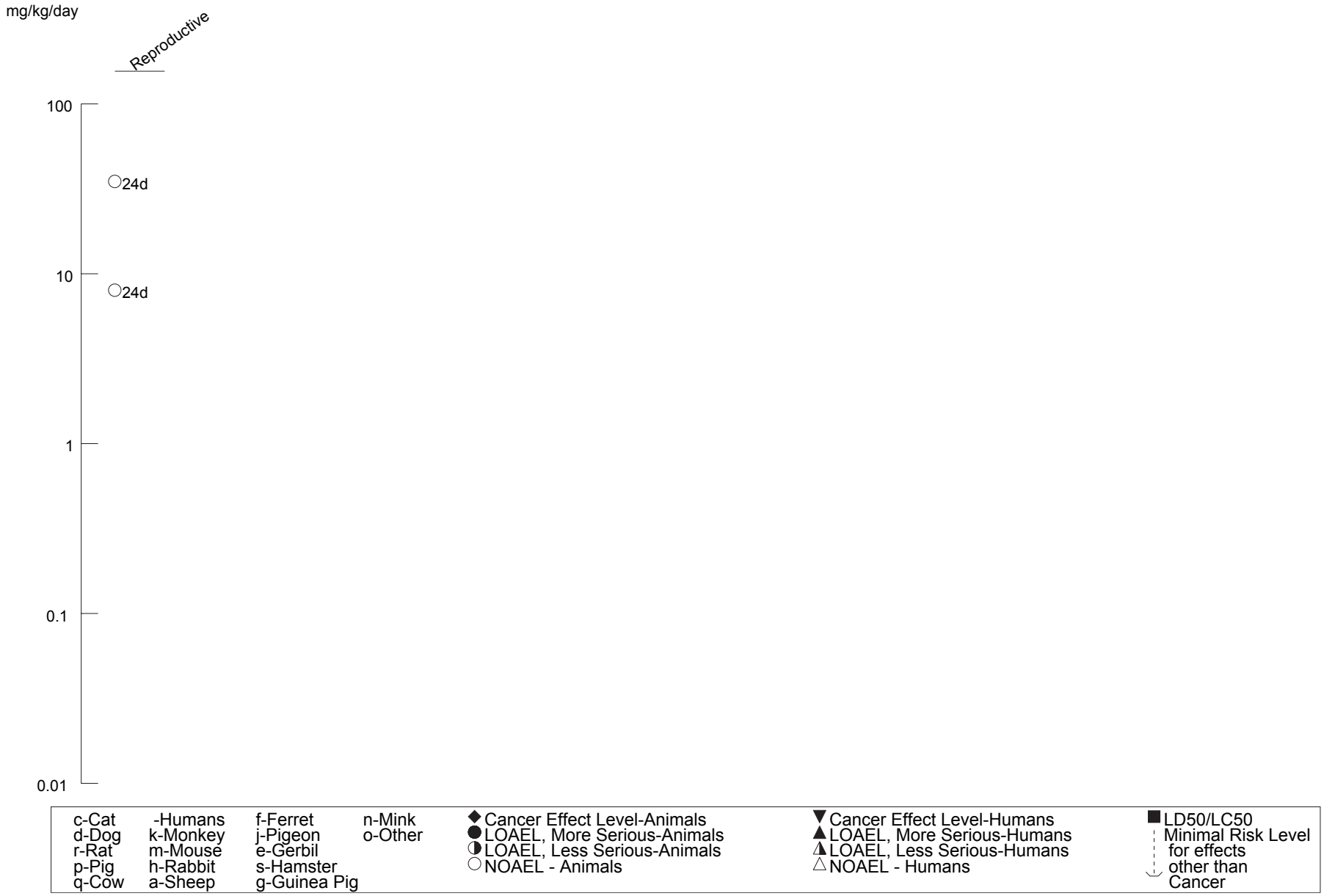


Table 3-5 Levels of Significant Exposure to Dimethylarsinic Acid - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Rat (Fischer- 344)	13 wk (F)				475 M (100% mortality during first 2 weeks of study)	Crown et al. 1987 DMA	
2	Rat (CD)	10 d Gd 7-16 1 x/d (GW)				60 F (67% mortality)	Rogers et al. 1981 DMA	
3	Mouse (ddY)	once (GW)				1200 M (LD50)	Kaise et al. 1989 DMA	
4	Mouse (CD-1)	10 d Gd 7-16 1 x/d (GW)				600 F (59% mortality)	Rogers et al. 1981 DMA	
Systemic								
5	Rat (Fischer- 344)	2 wk (F)	Renal		11 F (altered bladder cell surface characteristics)		Cohen et al. 2001 DMA	
			Bd Wt	11 F				
6	Rat (Fischer- 344)	13 wk (F)	Gastro		475 M (diarrhea and congestion and hemorrhagic contents in gastrointestinal tract in rats dying during first 2 weeks)		Crown et al. 1987 DMA	

Table 3-5 Levels of Significant Exposure to Dimethylarsinic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
7	Rat (Sprague-Dawley)	Gd 6-15 (GW)	Bd Wt	12 F	36 F (decreased maternal body weight gain)		Irvine et al. 2006 DMA	
8	Rat (CD)	10 d Gd 7-16 1 x/d (GW)	Bd Wt			40 F (27% decreased maternal weight gain)	Rogers et al. 1981 DMA	
9	Mouse (B6C3F1)	24 hr 1 or 2 x (GW)	Resp		720 F (decreased lung ODC)		Ahmad et al. 1999a DMA	
			Hepatic		720 F (decreased liver GSH, GSSG, CYP-450 and ODC; increased serum ALT)			
10	Mouse (ddY)	once (GW)	Resp			900 M (respiratory arrest)	Kaise et al. 1989 DMA	
			Gastro		1757 M (diarrhea, slight congestion of the intestion)			
11	Mouse (CD-1)	10 d Gd 7-16 1 x/d (GW)	Bd Wt			200 F (26% decreased maternal weight gain)	Rogers et al. 1981 DMA	
12	Dog (Beagle)	52 wk (C)	Gastro	6.5	16 (vomiting and diarrhea)		Zomber et al. 1989 DMA	

Table 3-5 Levels of Significant Exposure to Dimethylarsinic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
13	Rabbit (New Zealand)	Gd 7-19 (GW)	Gastro	12 F	48 F (fluid gastrointestinal tract contents)		Irvine et al. 2006 DMA	
			Bd Wt	12 F		48 F (maternal weight loss)		
Neurological								
14	Mouse (ddY)	once (GW)				1757 M (increased startle reflex; ataxia)	Kaise et al. 1989 DMA	
Developmental								
15	Rat (Sprague-Dawley)	GD6-15 (GW)			40 F (decreased fetal body weight)		Chernoff et al. 1990 DMA	
16	Rat (Sprague-Dawley)	Gd 6-15 (GW)		12	36 (decreases in number of live fetuses and fetal weight; increases in fetuses with diaphragmatic hernia; delayed ossification)		Irvine et al. 2006 DMA	
17	Rat (CD)	10 d Gd 7-16 1 x/d (GW)		15		30 (malformed palates in 15%)	Rogers et al. 1981 DMA	

Table 3-5 Levels of Significant Exposure to Dimethylarsinic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
18	Mouse (CD-1)	Gd 8 (GW)				1600 F (fetal deaths, decreased fetal weight, delayed ossification, skeletal malformations)	Kavlock et al. 1985 DMA	
19	Mouse (CD-1)	10 d Gd 7-16 1 x/d (GW)		200		400 (18% decrease in fetal weight, delayed ossification, cleft palate in 12/28; irregular palatine rugae in 4.8%)	Rogers et al. 1981 DMA	
20	Rabbit (New Zealand)	Gd 7-19 (GW)		12			Irvine et al. 2006 DMA	
INTERMEDIATE EXPOSURE								
Death								
21	Rat (Fischer- 344)	13 wk (F)				190 M (100% mortality during first 4 weeks of study)	Crown et al. 1987 DMA	
22	Rat (Fischer- 344)	4 wk 5 d/wk 1 x/d (G)				57 (50% survival in males; 20% survival in females)	Murai et al. 1993 DMA	

Table 3-5 Levels of Significant Exposure to Dimethylarsinic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
23	Rat (Fischer- 344)	8 wk (W)				17 M (10/10 died)	Wanibuchi et al. 1996 DMA	
Systemic								
24	Rat (Fischer- 344)	10 or 20 wk (F)	Renal	1 F	10 M (necrosis in bladder epithelium)		Arnold et al. 1999 DMA	
					[b] 5 F (increased kidney weight, calcification at corticomedullary junction; increased bladder weight and increased BrdU labelling in bladder epithelium)			
			Bd Wt	10				
25	Rat (Fischer- 344)	10 wk (F)	Renal		11 F (increased bladder and kidney weights, hyperplasia and necrosis of bladder epithelium)		Cohen et al. 2001 DMA	

Table 3-5 Levels of Significant Exposure to Dimethylarsinic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
26	Rat (Fischer- 344)	13 wk (F)	Resp	43.2 M			Crown et al. 1987 DMA	
			Cardio	43.2 M				
			Gastro	43.2 M	190 M (diarrhea and congestion and hemorrhagic contents in gastrointestinal tissues)			
			Hemato	0.44 F	4.5 F (decreased hemoglobin and erythrocyte levels)			
			Hepatic	23.5 M				
			Renal	0.4 M	4 M (increased urine volume and decreased specific gravity)			
			Endocr	0.4 M	4 M (hypertrophy of thyroid follicle epithelium)			
			Bd Wt	43.2 M				
27	Rat (Fischer- 344)	4 wk 5 d/wk 1 x/d (G)	Renal			57 (papillary necrosis and hyperplasia; cortical degeneration and necrosis)	Murai et al. 1993 DMA	
			Bd Wt		57 (decreased body weight)			

Table 3-5 Levels of Significant Exposure to Dimethylarsinic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
28	Rat (Sprague-Dawley)	10 wk pre-mating, gestation and lactation periods (F)	Resp	16.5			Rubin et al. 1989 DMA	
			Cardio	16.5				
			Hemato	0.34 M	2.3 M (decreased mean corpscular hemoglobin concentration)			
			Hepatic	16.5				
			Renal	16.5				
			Endocr	2.3 F	16.5 F (hypertrophy of thyroid follicle epithelium)			
			Bd Wt	16.5				
29	Rat (Sprague-Dawley)	42 d (F)	Hemato	3.7 M			Siewicki 1981 DMA	
			Hepatic	3.7 M				
			Renal	3.7 M				
			Bd Wt	3.7 M				

Table 3-5 Levels of Significant Exposure to Dimethylarsinic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
30	Dog (Beagle)	6 d/wk 52 wk (C)	Gastro	6.5	16 (vomiting and diarrhea)		Zomber et al. 1989 DMA	
			Hemato	16 M	40 M (decreased erythrocyte and increased leukocyte levels)			
Reproductive								
31	Rat (Sprague-Dawley)	10 wk pre-mating, gestation and lactation periods (F)		16.5			Rubin et al. 1989 DMA	
Cancer								
32	Mouse A/J	50 wk ad lib (W)				10.4 M (CEL: lung tumors)	Hayashi et al. 1998 DMA	

Table 3-5 Levels of Significant Exposure to Dimethylarsinic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
CHRONIC EXPOSURE								
Systemic								
33	Rat (Fischer- 344)	daily 2 yr (F)	Resp	7.8			Arnold et al. 2006 DMA	
			Cardio	7.8				
			Gastro	7.8				
			Hemato	7.8				
			Musc/skel	7.8				
			Hepatic	7.8				
			Renal	0.77 M	3.1 M (nephrocalcinosis)			
				0.77 F	3.1 F (urothelial vacular degeneration and hyperplasia of urothelial cells in urinary bladder)			
			Endocr	3.1	7.8 (hypertrophy of thyroid follicle epithelium)			
			Dermal	7.8				
			Ocular	7.8				
			Bd Wt	7.8				
34	Rat (Fischer- 344)	104 wk (W)	Renal	0.75 M	3.4 M (nodular or papillary hyperplasia in urinary bladder)		Wei et al. 1999, 2002 DMA	

Table 3-5 Levels of Significant Exposure to Dimethylarsinic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
35	Mouse (B6C3F1)	daily 2 yr (F)	Resp	94			Arnold et al. 2006 DMA	
			Cardio	94				
			Gastro	94				
			Hemato	94 F	94 F (decreased lymphocytes and increased monocytes)			
			Musc/skel	94				
			Hepatic	94				
			Renal	1.3[c] F	37 M (progressive glomerulonephropathy)			
					7.8[b] F (vacuolization of superficial cells of urotheliumin urinary bladder)			
			Dermal	94				
			Ocular	94				
			Bd Wt	94				

Table 3-5 Levels of Significant Exposure to Dimethylarsinic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
36	Dog (Beagle)	6 d/wk 52 wk (C)	Resp	40			Zomber et al. 1989 DMA	
			Cardio	40				
			Gastro	6.5	16 (vomiting and diarrhea)			
			Hepatic	40				
			Renal	40				
Reproductive								
37	Rat (Fischer- 344)	daily 2 yr (F)		7.8			Arnold et al. 2006 DMA	Histological examination of reproductive tissues.
38	Mouse (B6C3F1)	daily 2 yr (F)		94			Arnold et al. 2006 DMA	Histological examination of reproductive tissues.
Cancer								
39	Rat (Fischer- 344)	daily 2 yr (F)				7.8 (CEL: urothelial cell papillomas and carcinomas in urinary bladder)	Arnold et al. 2006 DMA	
40	Rat (Fischer- 344)	104 wk (W)				3.4 M (CEL: urinary bladder tumors)	Wei et al. 1999, 2002 DMA	

Table 3-5 Levels of Significant Exposure to Dimethylarsinic Acid - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
41	Mouse knockout	continuous 18 mo (W)				11.8 M (CEL)	Salim et al. 2003 DMA	

a The number corresponds to entries in Figure 3-5.

b Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 3-5. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

c The chronic-duration oral MRL of 0.02 mg DMA/kg/day was calculated using a benchmark dose analysis. The BMDL10 of 1.80 mg DMA/kg/day was divided by an uncertainty factor of 100 (10 to account for extrapolation from animals to humans and 10 for human variability)

ad lib = ad libitum; ALT = alanine aminotransferase; Bd Wt = body weight; BrdU = bromodeoxyuridine; (C) = capsule; Cardio = cardiovascular; CEL = cancer effect level; CYP = cytochrome p; d= day(s); DMA = dimethylarsinic acid; Endocr = endocrine; (F) = feed; F = female; (G) = gavage; Gastro = gastrointestinal; Gd = gestation day; GSH = reduced glutathione; GSSG = oxidized glutathione; (GW) = gavage in water; Hemato = hematological; hr = hour(s); LD50 = lethal dose, 50% kill; ODC = ornithine decarboxylase; LOAEL = lowest-observable-adverse-effect level; M = male; mo = month; Musc/skel = musculoskeletal; NOAEL = no-observable-adverse-effect level; Resp = respiratory; (W) = drinking water; wk = week(s); x = time(s); yr = year(s)

Figure 3-5 Levels of Significant Exposure to Dimethylarsinic Acid - Oral

Acute (≤14 days)

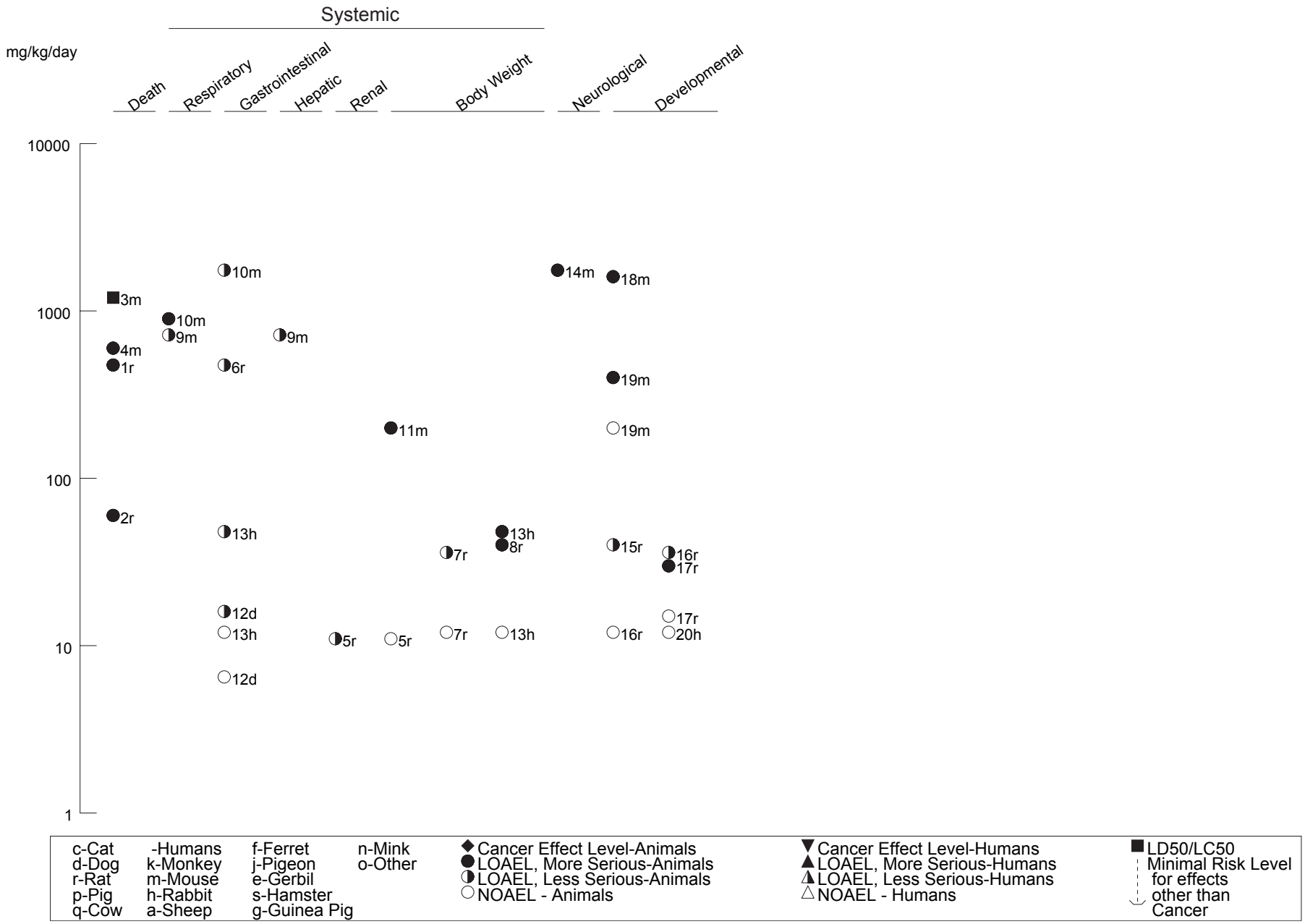
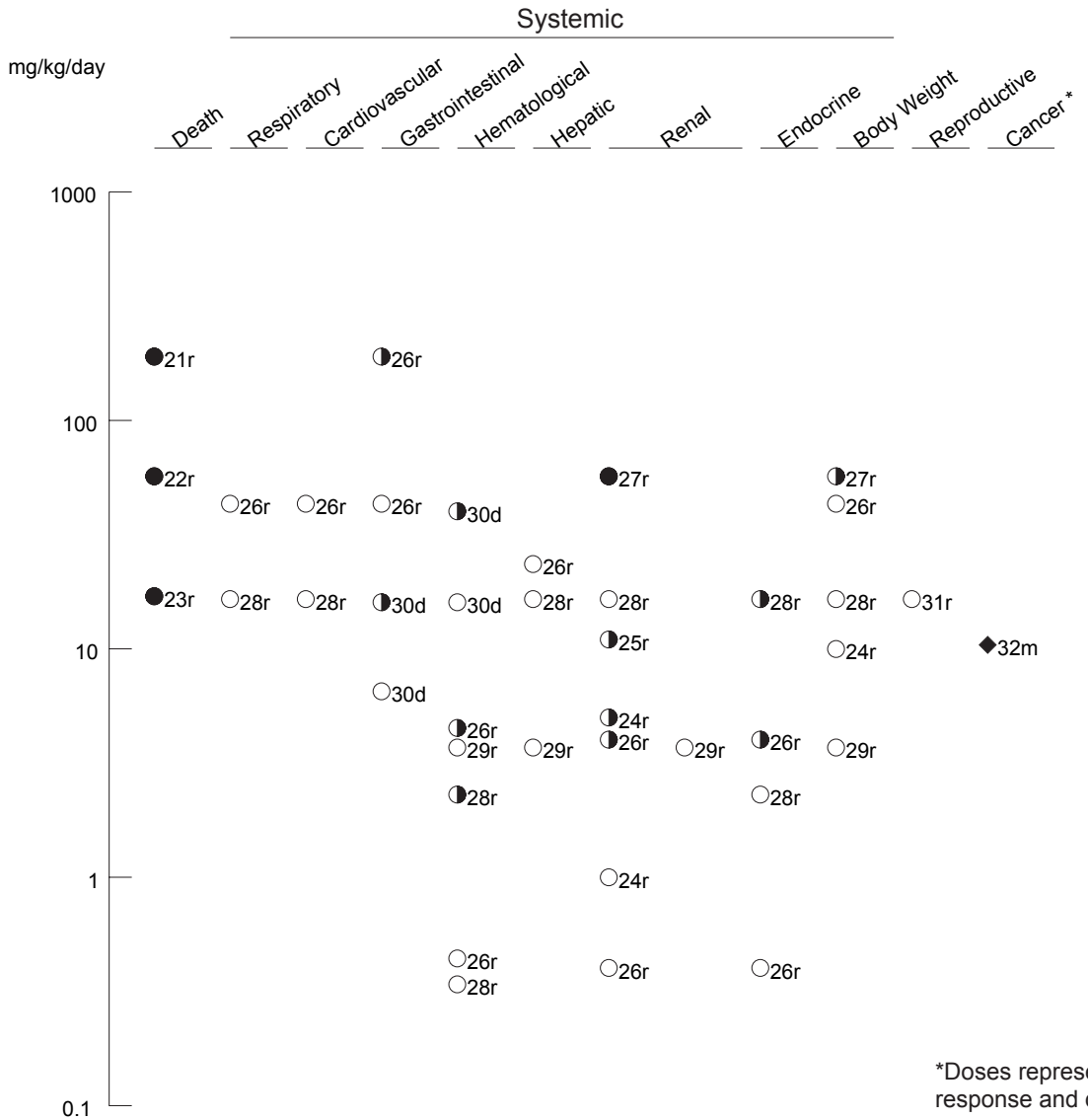


Figure 3-5 Levels of Significant Exposure to Dimethylarsinic Acid - Oral *(Continued)*

Intermediate (15-364 days)

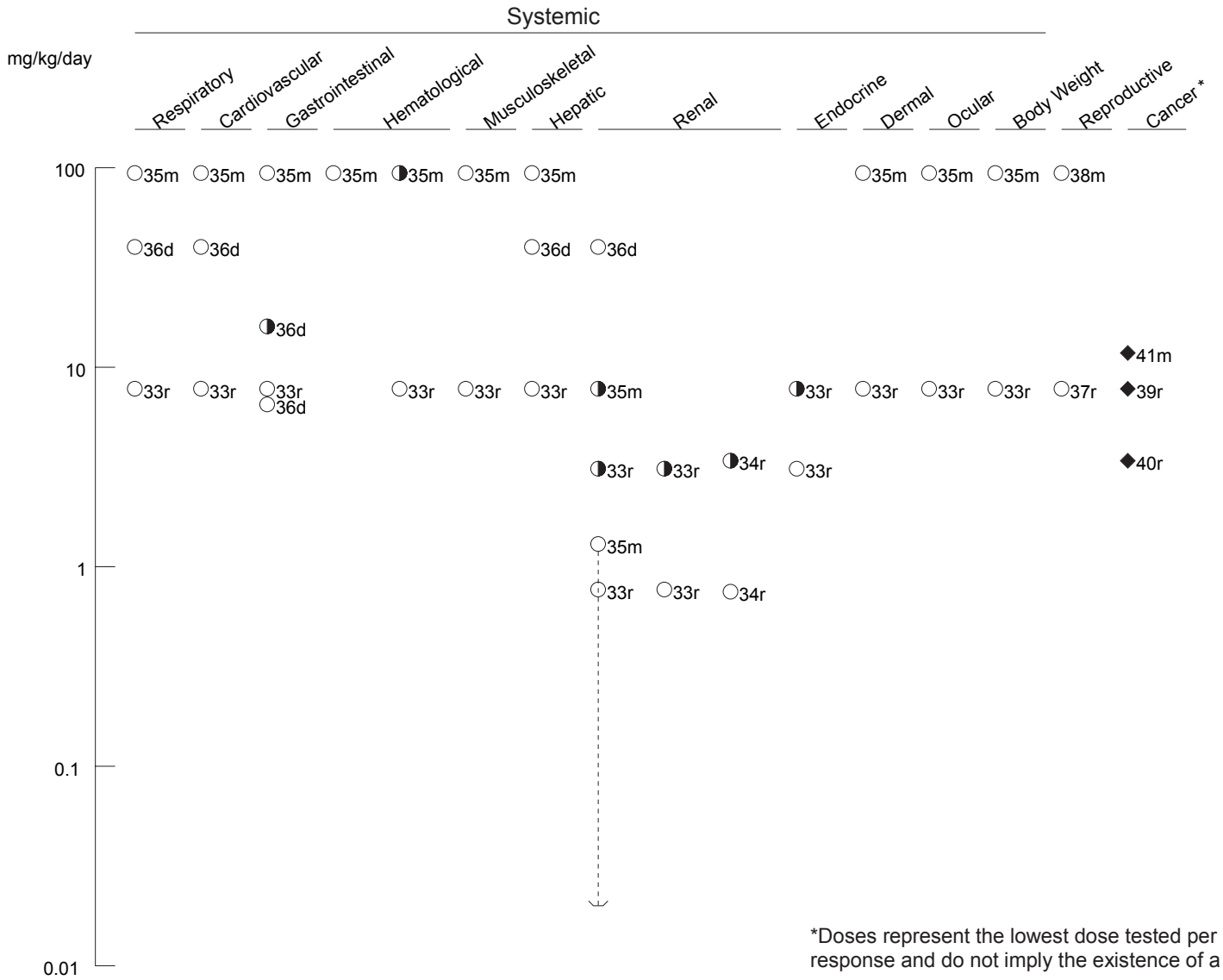


*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint.

c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level for effects other than Cancer
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◭ LOAEL, Less Serious-Humans	
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	
q-Cow	a-Sheep	g-Guinea Pig				

Figure 3-5 Levels of Significant Exposure to Dimethylarsinic Acid - Oral *(Continued)*

Chronic (≥365 days)



*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint.

c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level for effects other than Cancer
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◭ LOAEL, Less Serious-Humans	
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	
q-Cow	a-Sheep	g-Guinea Pig				

Table 3-6 Levels of Significant Exposure to Roxarsone - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Rat (Holtzman)	once (GW)				155 (LD50)	Kerr et al. 1963 ROX	
2	Rat (Fischer- 344)	once (GO)				150 M (5/5 died) 81[b] F (LD50)	NTP 1989b ROX	
3	Rat (Fischer- 344)	14 d (F)				128[b] M (3/5 died) 144 F (5/5 died)	NTP 1989b ROX	
4	Mouse (B6C3F1)	once (GO)				300 M (5/5 died) 244[b] F (LD50)	NTP 1989b ROX	
5	Mouse (B6C3F1)	14 d (F)				168 F (5/5 died)	NTP 1989b ROX	
Systemic								
6	Rat (Fischer- 344)	14 d (F)	Bd Wt	16 M	32 M (22% reduced body weight)		NTP 1989b ROX	
7	Mouse (B6C3F1)	14 d (F)	Bd Wt	84		168 (34% decrease in body weight)	NTP 1989b ROX	
Neurological								
8	Rat (Fischer- 344)	14 d (F)		16 M	32 M (slight inactivity)		NTP 1989b ROX	

Table 3-6 Levels of Significant Exposure to Roxarsone - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
9	Mouse (B6C3F1)	14 d (F)		20	42 (slight inactivity; ruffled fur)		NTP 1989b ROX	
10	Pig (Landrace)	30 d ad lib (F)				6.3 (muscle tremors and clonic convulsions)	Rice et al. 1985; Kennedy et al. 1986 ROX	
INTERMEDIATE EXPOSURE								
Death								
11	Rat (Holtzman)	13 wk (F)				20 (10/12 died)	Kerr et al. 1963 ROX	
12	Rat (Fischer- 344)	13 wk ad lib (F)				64 M (3/10 died)	NTP 1989b ROX	
13	Mouse (B6C3F1)	13 wk ad lib (F)				136 (6/10 males and 8/10 females died)	NTP 1989b ROX	

Table 3-6 Levels of Significant Exposure to Roxarsone - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Systemic								
14	Rat (Fischer- 344)	31 or 90 d ad lib (F)	Hemato	32 M			NTP 1989b ROX	
			Hepatic	9 F	36 F (decreased absolute and relative liver weight)			
			Renal	8 M	32 M (increased kidney weight; minimal tubular degeneration)			
			Bd Wt	8 M		32 M (27% decrease in body weight)		

Table 3-6 Levels of Significant Exposure to Roxarsone - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
15	Rat (Fischer- 344)	13 wk ad lib (F)	Resp	64 M			NTP 1989b ROX	
			Cardio	64 M				
			Gastro	64 M				
			Musc/skel	64 M				
			Hepatic	4 M	8 M (increased relative liver weight)			
			Renal	16 M	32 M (interstitial inflammation, focal regenerative hyperplasia of tubular cell epithelium and mineralization)			
			Endocr	64 M				
			Dermal	64 M				
			Bd Wt	8 M	16 M (14% decreased body weight)	32 M (26% decreased body weight)		

Table 3-6 Levels of Significant Exposure to Roxarsone - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
16	Mouse (B6C3F1)	13 wk ad lib (F)	Cardio	136			NTP 1989b ROX	
			Gastro	136				
			Musc/skel	136				
			Hepatic	136				
			Renal	136				
			Endocr	136				
			Dermal	136				
			Bd Wt		136 (18% decreased body weight in males; 11% decreased body weight in females)			
17	Mouse (B6C3F1)	29 or 91 d ad lib (F)	Hemato	68			NTP 1989b ROX	
			Hepatic	68				
			Renal	68				
Neurological								
18	Rat (Fischer- 344)	13 wk ad lib (F)		32 M		64 M (trembling, ataxia, hyperexcitability, slight inactivity, ruffled fur)	NTP 1989b ROX	

Table 3-6 Levels of Significant Exposure to Roxarsone - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form	Comments
19	Pig	28 d (F)				10	(muscle tremors)	Edmonds and Baker 1986 ROX	
20	Pig (Landrace)	30 d ad lib (F)				6.3	(paraplegia, myelin degeneration in spinal cord, peripheral nerves, optic nerve)	Rice et al. 1985; Kennedy et al. 1986 ROX	
CHRONIC EXPOSURE									
Systemic									
21	Rat (Fischer- 344)	103 wk ad lib (F)	Resp	4				NTP 1989b ROX	
			Cardio	4					
			Gastro	4					
			Musc/skel	4					
			Hepatic	4					
			Renal	4					
			Endocr	4					
			Dermal	4					
			Ocular	4					
			Bd Wt	4					
22	Mouse (Fischer- 344)	103 wk ad lib (F)	Resp	43 M				NTP 1989b ROX	
			Cardio	43 M					
			Gastro	43 M					
			Musc/skel	43 M					
			Hepatic	43 M					

Table 3-6 Levels of Significant Exposure to Roxarsone - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
			Renal	43 M				
			Endocr	43 M				
			Dermal	43 M				
			Ocular	43 M				
			Bd Wt	43 F				

a The number corresponds to entries in Figure 3-6.

b Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 3-6. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

ad lib = ad libitum; Bd Wt = body weight; Cardio = cardiovascular; d = day(s); Endocr = endocrine; (F) = feed; F = Female; Gastro = gastrointestinal; (GO) = gavage in oil; (GW) = gavage in water; Hemato = hematological; LC50 = lethal concentration, 50% kill, LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; occup = occupational; Resp = respiratory; wk = week(s)

Figure 3-6 Levels of Significant Exposure to Roxarsone - Oral

Acute (≤14 days)

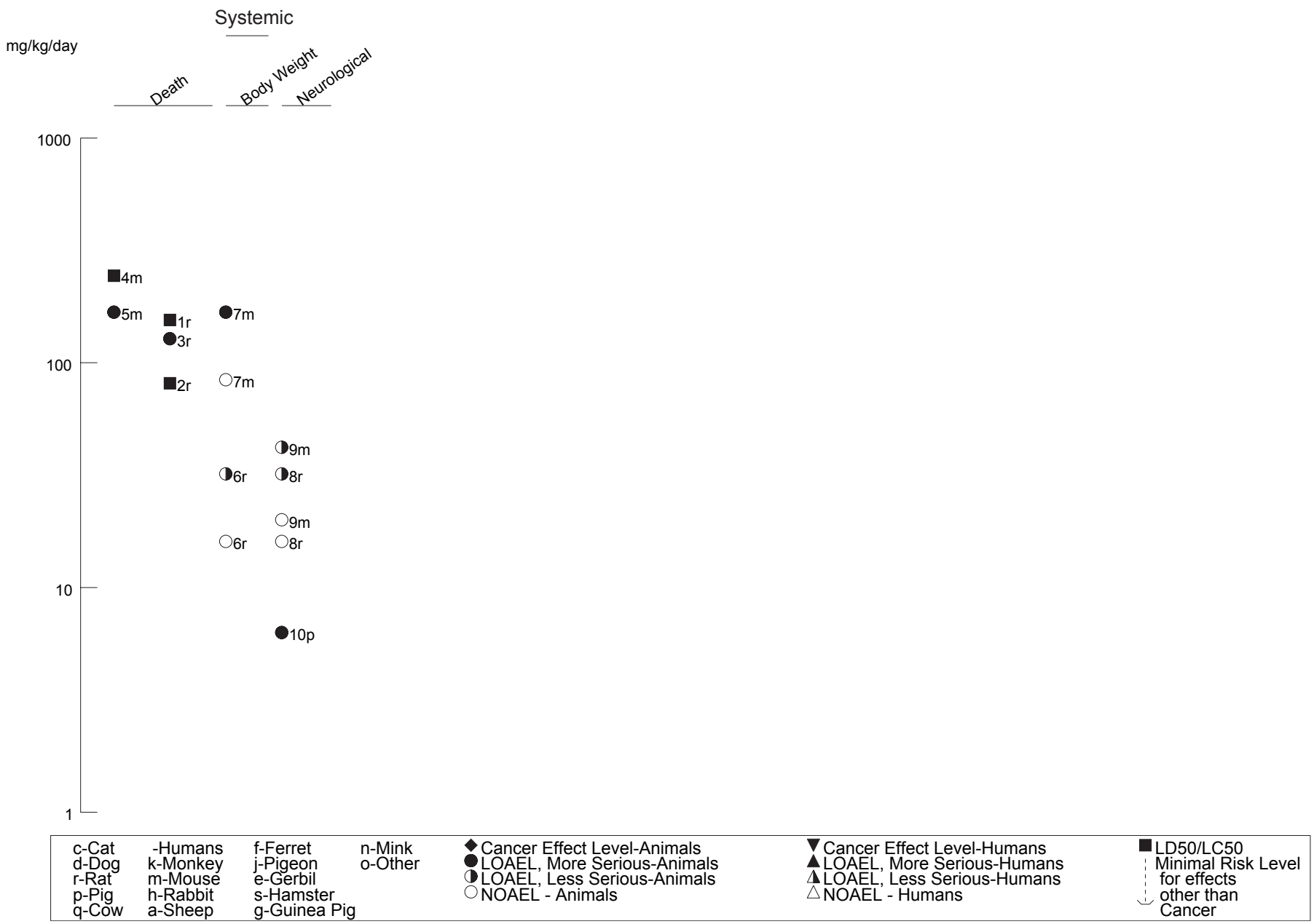
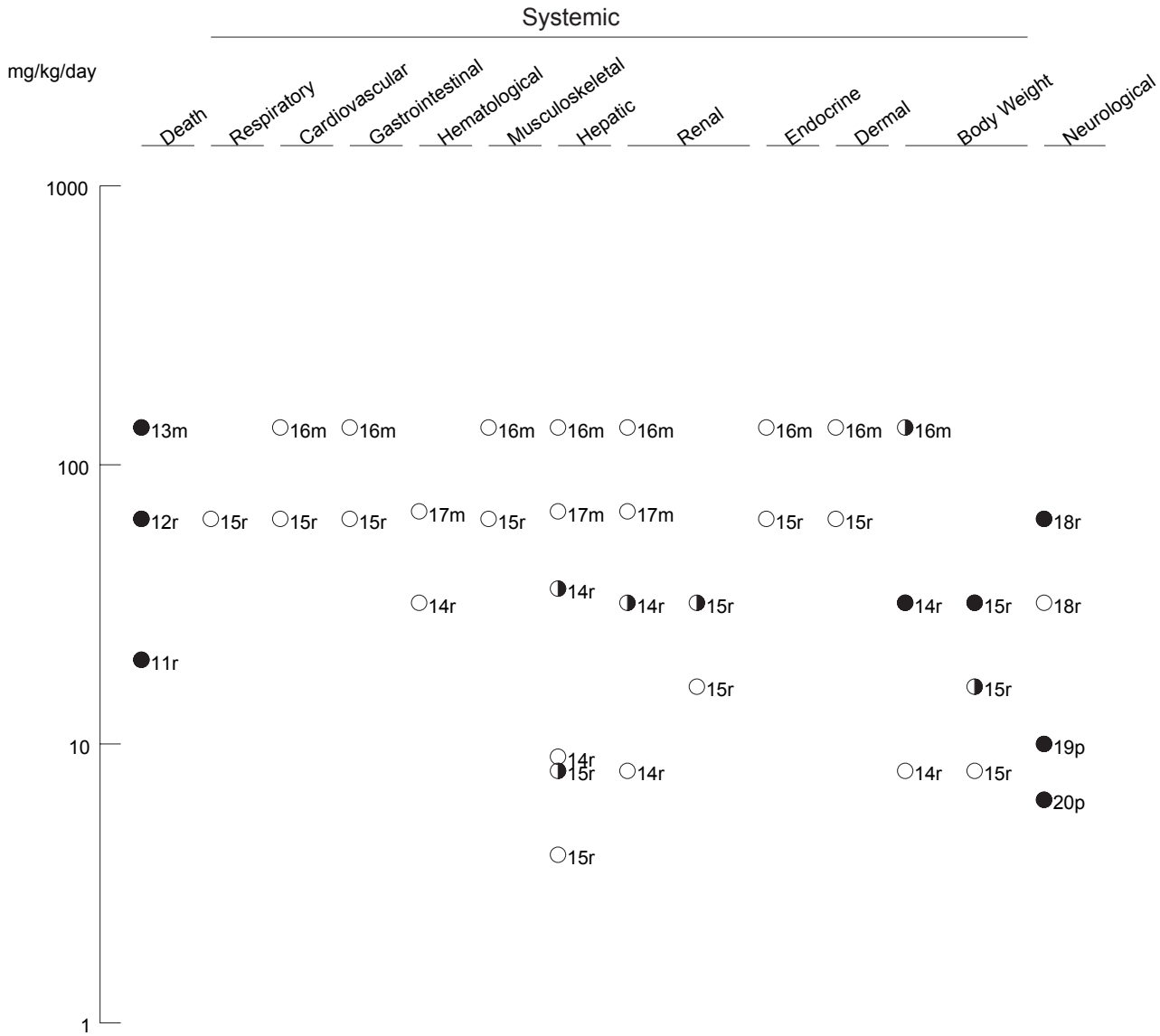


Figure 3-6 Levels of Significant Exposure to Roxarsone - Oral *(Continued)*

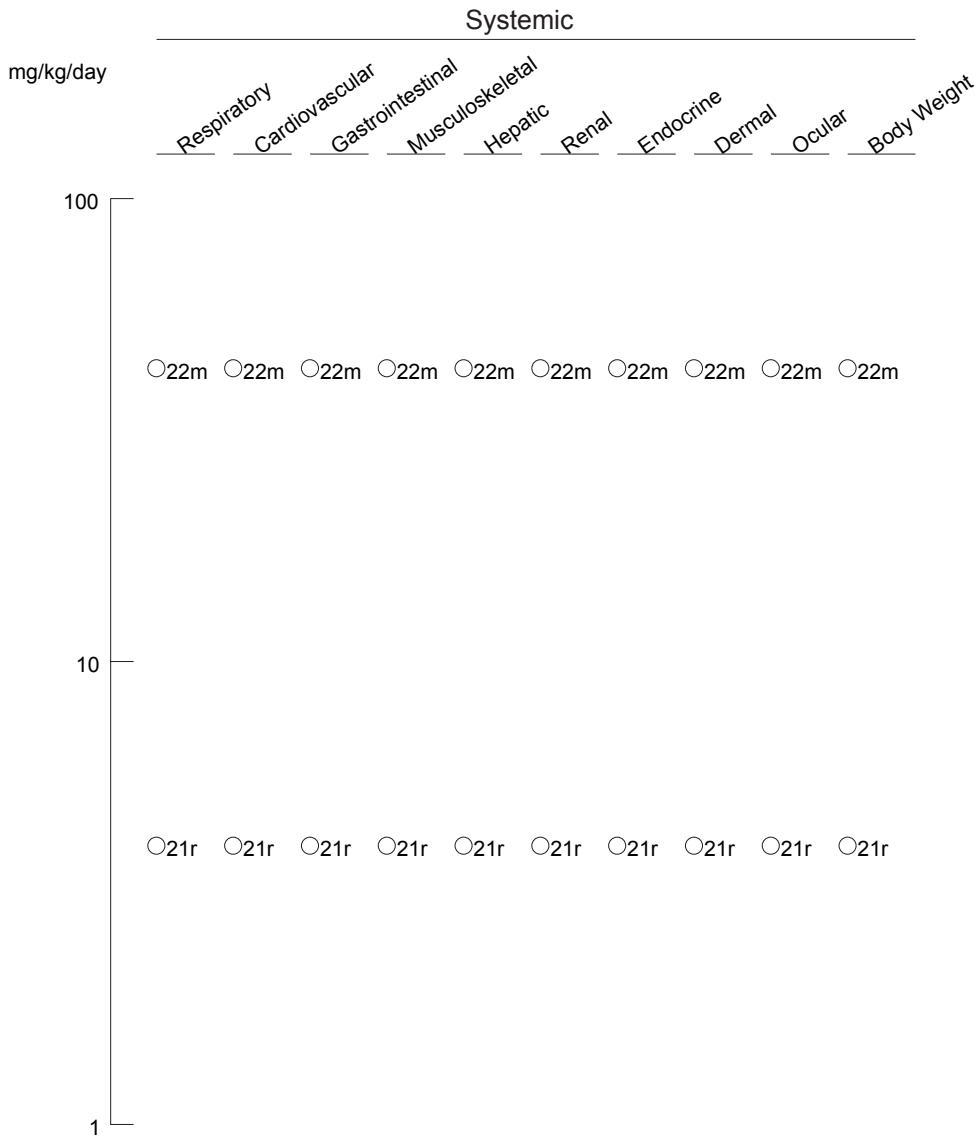
Intermediate (15-364 days)



c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level for effects other than Cancer
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◭ LOAEL, Less Serious-Humans	
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	
q-Cow	a-Sheep	g-Guinea Pig				

Figure 3-6 Levels of Significant Exposure to Roxarsone - Oral *(Continued)*

Chronic (≥365 days)



c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level for effects other than Cancer
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◭ LOAEL, Less Serious-Humans	
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	
q-Cow	a-Sheep	g-Guinea Pig				

Available LD_{50} values for arsenate and arsenite in rats and mice range from 15 to 175 mg As/kg (Dieke and Richter 1946; Gaines 1960; Harrisson et al. 1958; Kaise et al. 1985). The variability can be attributed to differences based on species, strain, specific route of exposure (feed vs. gavage), specific compound tested, and testing laboratory. Most deaths occurred within 1 day of exposure, but details regarding cause of death were not generally reported. Seven of 25 pregnant rats given a single gavage dose of 23 mg As/kg as arsenic trioxide on day 9 of gestation died soon after dosing, while no deaths occurred at doses of 4–15 mg As/kg (Stump et al. 1999). Data on lethality from repeated exposure studies in animals are relatively sparse. Seven of 20 pregnant rabbits died from repeated gavage doses of 1.5 mg As/kg/day as arsenic acid during gestation, while none died at 0.1–0.4 mg As/kg/day (Nemec et al. 1998). Chronic studies observed treatment-related mortality in monkeys exposed to 3 mg As/kg/day as arsenate (Heywood and Sortwell 1979), dogs exposed to 2.4 mg As/kg/day as arsenite or arsenate (Byron et al. 1967), mice exposed to 1 mg As/kg/day as arsenite (Schroeder and Balassa 1967), and rats exposed to 30 mg As/kg/day as lead arsenate (Kroes et al. 1974).

Reliable LOAEL and LD_{50} values for lethality from oral exposure to inorganic arsenicals in each species and duration category are recorded in Table 3-3 and plotted in Figure 3-3.

Organic Arsenicals. No studies were located regarding death in humans after oral exposure to organic arsenicals, but the acute lethality of MMA, DMA, and roxarsone have been investigated in several animal studies. The LD_{50} values for MMA (including MSMA), DMA, and roxarsone are 102–3,184 mg/kg MMA or MSMA (Gur and Nyska 1990; Jaghabir et al. 1988; Kaise et al. 1989), 1,200 mg DMA/kg/day (Kaise et al. 1989), and 14.2–69.5 mg DMA/kg/day (Kerr et al. 1963; NTP 1989b), respectively. The cause of death was not investigated in any of these studies. Intermediate-duration exposure to MMA, DMA, or roxarsone resulted in increased mortality in laboratory animals exposed to 106.9 mg MMA/kg/day (Arnold et al. 2003), 17–190 mg DMA/kg/day (Crown et al. 1987; Murai et al. 1993; Wanibuchi et al. 1996) or 20–64 mg/kg/day roxarsone (Edmonds and Baker 1986; Kerr et al. 1963; NTP 1989b), respectively. Increased mortality was also observed in rats chronically exposed to 72.4 mg MMA/kg/day (Arnold et al. 2003).

3.2.2.2 Systemic Effects

The highest NOAEL values and all reliable LOAEL values for systemic effects from oral exposure in each species and duration category are recorded in Table 3-3 and plotted in Figure 3-3. Similar data for

oral exposure to MMA, DMA, and roxarsone are shown in Tables 3-4, 3-5, and 3-6, and shown in Figures 3-4, 3-5, and 3-6, respectively.

Respiratory Effects.

Inorganic Arsenicals. Serious respiratory effects, including respiratory distress, hemorrhagic bronchitis, and pulmonary edema, have been reported in some cases of acute oral arsenic poisoning at doses of 8 mg As/kg and above (e.g., Civantos et al. 1995; Fincher and Koerker 1987; Levin-Scherz et al. 1987; Moore et al. 1994b; Quatrehomme et al. 1992). These effects may be secondary to injury to the pulmonary vasculature (see Cardiovascular Effects, below). In addition, bronchitis and sequelae (bronchiectasis, bronchopneumonia) have been observed in patients and at autopsy in some chronic poisoning cases (Guha Mazumder et al. 2005; Milton and Rahman 2002; Rosenberg 1974; Tsai et al. 1999; Zaldívar 1974; Zaldívar and Guillier 1977). Bronchopneumonia secondary to arsenic-induced bronchitis was considered to be the cause of death in one young child who died after several years of exposure to an average dose of 0.08 mg As/kg/day (Zaldívar and Guillier 1977). Decrements in lung function, measured as decreased FEV_1, FVC, and $FEF_{25–75}$ have also been reported in subjects exposed to 0.1–0.5 mg As/L in the drinking water and exhibiting skin lesions (von Ehrenstein et al. 2005). In general, however, respiratory effects have not been widely associated with repeated oral ingestion of low arsenic doses. Nevertheless, a few studies have reported minor respiratory symptoms, such as cough, sputum, rhinorrhea, and sore throat, in people with repeated oral exposure to 0.03–0.05 mg As/kg/day (Ahmad et al. 1997; Mizuta et al. 1956).

There are few data regarding respiratory effects in animals following acute oral exposure to inorganic arsenic. An infant Rhesus monkey that died after 7 days of oral exposure to a complex arsenate salt at a dose of 3 mg As/kg/day exhibited bronchopneumonia with extensive pulmonary hemorrhage, edema, and necrosis (Heywood and Sortwell 1979). Two other monkeys in this treatment group survived a 1-year exposure period and had no gross or microscopic pulmonary lesions at sacrifice. Increased relative lung weights were seen in rats exposed to 6.66 mg As/kg/day as sodium arsenite 5 days/week for 12 weeks (Schulz et al. 2002). Chronic oral studies in dogs and rats treated with arsenate or arsenite failed to find respiratory lesions (Byron et al. 1967; Kroes et al. 1974; Schroeder et al. 1968).

One study utilizing gallium arsenide included limited investigation of respiratory function. Respiration rate was significantly decreased in rats following ingestion of a single dose of gallium arsenide at 1,040 mg As/kg, but was unaffected at a dose of 520 mg As/kg (Flora et al. 1997a). Respiration rate was measured 1, 7, and 15 days after dosing, but the decrease was most noticeable after 15 days.

Organic Arsenicals. No respiratory effects were noted after acute human ingestion of 1,714 mg MSMA/kg (Shum et al. 1995). Mice exhibited respiratory arrest after a single oral dose of 1,800 mg MMA/kg (Kaise et al. 1989) or 900 mg DMA/kg (Kaise et al. 1989) and lung ornithine decarboxylase activity was reduced after ingestion of one or two doses of 720 mg DMA/kg (Ahmad et al. 1999a). Localized lung hemorrhage was observed in dogs after a single oral dose of 14.2 mg/kg roxarsone in a capsule (Kerr et al. 1963). No respiratory effects were seen after intermediate or chronic exposure of rats, mice, or dogs exposed to 35 mg MMA/kg/day (Waner and Nyska 1988), 7.8–94 mg DMA/kg/day (Arnold et al. 2006; Crown et al. 1987; Rubin et al. 1989; Zomber et al. 1989), or 4–136 mg/kg/day roxarsone (NTP 1989b).

Cardiovascular Effects.

Inorganic Arsenicals. A number of studies in humans indicate that arsenic ingestion may lead to serious effects on the cardiovascular system. Characteristic effects on the heart from both acute and long-term exposure include altered myocardial depolarization (prolonged QT interval, nonspecific ST segment changes) and cardiac arrhythmias (Cullen et al. 1995; Glazener et al. 1968; Goldsmith and From 1986; Heyman et al. 1956; Little et al. 1990; Mizuta et al. 1956; Moore et al. 1994b; Mumford et al. 2007). A significant dose-related increase in the prevalence of cardiac electrophysiologic abnormalites was observed in residents of Inner Mongolia, China; the incidences of QT prolongation were observed in 3.9, 11.1, and 20.6% of the residents with drinking water levels of <21, 110–300, and 430–690 μg/L, respectively (Mumford et al. 2007). Hypertrophy of the ventricular wall was observed at autopsy after acute exposure to 93 mg of arsenic (Quatrehomme et al. 1992). Long-term, low-level exposures may also lead to damage to the vascular system. The most dramatic example of this is "Blackfoot Disease," a condition that is endemic in an area of Taiwan where average drinking water levels of arsenic range from 0.17 to 0.80 ppm (Tseng 1977), corresponding to doses of about 0.014–0.065 mg As/kg/day (IRIS 2007). The disease is characterized by a progressive loss of circulation in the hands and feet, leading ultimately to necrosis and gangrene (Chen et al. 1988b; Ch’i and Blackwell 1968; Tseng 1977, 1989; Tseng et al. 1968, 1995, 1996). Several researchers have presented evidence that other factors besides arsenic (e.g., other water contaminants, dietary deficits) may play a role in the etiology of this disease (Ko 1986; Lu et al. 1990; Yu et al. 1984). While this may be true, the clear association between the occurrence of Blackfoot Disease and the intake of elevated arsenic levels indicates that arsenic is at least a contributing factor. The results of a recent study suggested that individuals with a lower capacity to methylate

inorganic arsenic to DMA have a higher risk of developing peripheral vascular disease in the Blackfoot Disease-hyperendemic area in Taiwan (Tseng et al. 2005).

Arsenic exposure in Taiwan has also been associated with an increased incidence of cerebrovascular and microvascular diseases (Chiou et al. 1997; Wang et al. 2002, 2003) and ischemic heart disease (Chang et al. 2004; Chen et al. 1996; Hsueh et al. 1998b; Tsai et al. 1999; Tseng et al. 2003). Moreover, effects of arsenic on the vascular system have also been reported in a number of other populations. For example, hypertension, defined as a systolic blood pressure of ≥140 mm Hg in combination with a diastolic blood pressure of ≥90 mm Hg, was associated with estimated lifetime doses of approximately 0.055 mg As/kg/day (0.25 mg/L in water) in a study of people in Bangladesh (Rahman et al. 1999); no significant association was found with estimated doses of 0.018 mg As/kg/day (0.75 mg/L in water). Wang et al. (2003) found an increased incidence of microvascular and macrovascular disease among subjects in Taiwan living in an arseniasis-endemic area in which the water of artesian wells had arsenic concentrations >0.35 mg/L (estimated doses of >0.03 mg As/kg/day). An additional study of Taiwanese subjects reported a significant increase in incidence of hypertension associated with concentrations of arsenic in the water >0.7 mg/L (estimated doses of >0.06 mg As/kg/day) (Chen et al. 1995). Studies in Chile indicate that ingestion of 0.6–0.8 ppm arsenic in drinking water (corresponding to doses of 0.02–0.06 mg As/kg/day, depending on age) increases the incidence of Raynaud's disease and of cyanosis of fingers and toes (Borgoño and Greiber 1972; Zaldívar 1974, 1977; Zaldívar and Guillier 1977). Autopsy of five children from this region who died of apparent arsenic toxicity showed a marked thickening of small and medium sized arteries in tissues throughout the body, especially the heart (Rosenberg 1974). In addition, cardiac failure, arterial hypotension, myocardial necrosis, and thrombosis have been observed in children who died from chronic arsenic ingestion (Zaldívar 1974), as well as adults chronically exposed to arsenic (Dueñas et al. 1998). Likewise, thickening and vascular occlusion of blood vessels were noted in German vintners exposed to arsenical pesticides in wine and in adults who drank arsenic-contaminated drinking water (Roth 1957; Zaldívar and Guillier 1977). A survey of Wisconsin residents using private wells for their drinking water found that residents exposed for at least 20 years to water concentrations of >10 μg As/L had increased incidences of cardiac bypass surgery, high blood pressure, and circulatory problems as compared with residents exposed to lower arsenic concentrations (Zierold et al. 2004). Similarly, Lewis et al. (1999) reported increased mortality from hypertensive heart disease in both men and women among a cohort exposed to arsenic in their drinking water in Utah, as compared with the general population of Utah. Limitations in the study included lack of evaluation of smoking as a confounder and of other dietary sources of arsenic, and the lack of a dose-response for hypertensive heart disease. Another ecological study (Engel and Smith 1994) found significant increases in deaths from

arteriosclerosis, aortic aneurysm, and all other diseases of the arteries, arterioles, and capillaries among U.S. residents with arsenic drinking waters of >20 μg/L; the increase in deaths from congenital anomalies of the heart and other anomalies of the circulatory system also observed in this subpopulation limits the interpretation of the findings.

Similar alterations in vascular reactivity have been noted in rats given repeated oral doses of arsenic trioxide (11 mg As/kg/day) for several weeks (Bekemeier and Hirschelmann 1989), although no histological effects could be detected in the hearts of rats or dogs exposed to up to 30 mg As/kg/day as arsenate or arsenite for 2 years (Byron et al. 1967; Kroes et al. 1974; Schroeder et al. 1968). Acute exposure of rats to gallium arsenide at a dose of 1,040 mg As/kg resulted in an increase in blood pressure and heart rate, while 520 mg As/kg had no effect (Flora et al. 1997a). Guinea pigs exposed to arsenic trioxide for 1 day (0, 7.6, 22.7, or 37.9 mg As/kg) or 8 days (0 or 3.8 mg As/kg/day) showed prolongation of the cardiac QT interval and action potential duration (Chiang et al. 2002).

Organic Arsenicals. No adverse cardiovascular effects were noted after acute human ingestion of 1,714 mg MSMA/kg (Shum et al. 1995). However, sinus tachycardia was noted after acute ingestion of 73 mg DMA/kg (as dimethyl arsenic acid and dimethyl arsenate) (Lee et al. 1995). No cardiovascular effects were seen after intermediate or chronic exposure of laboratory animals to 35–67.1 mg MMA/kg/day (Arnold et al. 2003; Waner and Nyska 1988), 7.8–94 mg DMA/kg/day (Arnold et al. 2006; Crown et al. 1987; Rubin et al. 1989; Zomber et al. 1989), or 4–136 mg/kg/day roxarsone (NTP 1989b).

Gastrointestinal Effects.

Inorganic Arsenicals. Clinical signs of gastrointestinal irritation, including nausea, vomiting, diarrhea, and abdominal pain, are observed in essentially all cases of short-term high-dose exposures to inorganic arsenic (e.g., Armstrong et al. 1984; Bartolome et al. 1999; Campbell and Alvarez 1989; Chakraborti et al. 2003a; Cullen et al. 1995; Fincher and Koerker 1987; Goebel et al. 1990; Kingston et al. 1993; Levin-Scherz et al. 1987; Lugo et al. 1969; Moore et al. 1994b; Muzi et al. 2001; Uede and Furukawa 2003; Vantroyen et al. 2004). Similar signs are also frequently observed in groups or individuals with longer-term, lower-dose exposures (e.g., Borgoño and Greiber 1972; Cebrián et al. 1983; Franzblau and Lilis 1989; Guha Mazumder et al. 1988, 1998a; Haupert et al. 1996; Holland 1904; Huang et al. 1985; Mizuta et al. 1956; Nagai et al. 1956; Silver and Wainman 1952; Wagner et al. 1979; Zaldívar 1974), but effects are usually not detectable at exposure levels below about 0.01 mg As/kg/day (Harrington et al. 1978; Valentine et al. 1985). These symptoms generally decline within a short time after exposure ceases.

Gastrointestinal irritation symptoms form the basis (in part) for the acute oral MRL of 0.005 mg/kg/day for inorganic arsenic, as described in footnote b in Table 3-3. More severe symptoms (hematemesis, hemoperitoneum, gastrointestinal hemorrhage, and necrosis) have been reported in some cases with acute exposure to 8 mg As/kg or more (Civantos et al. 1995; Fincher and Koerker 1987; Levin-Scherz et al. 1987; Quatrehomme et al. 1992), and also in some people with long-term ingestion of 0.03–0.05 mg As/kg/day as a medicinal preparation (Lander et al. 1975; Morris et al. 1974).

Clinical signs of gastrointestinal irritation were observed in monkeys and rats given repeated oral doses of arsenic (6 and 11 mg As/kg/day, respectively) for 2 weeks (Bekemeier and Hirschelmann 1989; Heywood and Sortwell 1979). Hemorrhagic gastrointestinal lesions have also been reported in animal studies. A monkey that died after repeated oral treatment with 6 mg As/kg/day for approximately 1 month was found to have acute inflammation and hemorrhage of the small intestine upon necropsy (Heywood and Sortwell 1979). This lesion was not found in other monkeys that died in this study, or in the survivors. Two pregnant mice that died after repeated gavage treatment with 24 mg As/kg/day as arsenic acid had hemorrhagic lesions in the stomach (Nemec et al. 1998). Gross gastrointestinal lesions (stomach adhesions, eroded luminal epithelium in the stomach) were seen frequently in rats treated by gavage with 8 mg As/kg/day as arsenic trioxide starting before mating and continuing through the end of gestation (Holson et al. 2000). The lesions were not found in rats treated with 4 mg As/kg/day in this study. No histological evidence of gastrointestinal injury was detected in rats exposed to arsenate or arsenite in the feed for 2 years at doses up to 30 mg As/kg/day, but dogs fed a diet containing 2.4 mg As/kg/day as arsenite for 2 years had some bleeding in the gut (Byron et al. 1967; Kroes et al. 1974).

Organic Arsenicals. Vomiting was noted after ingestion of 793 mg/kg arsenic (as monosodium methanearsenate) in a suicide attempt (Shum et al. 1995). Ingestion of 78 mg DMA/kg (as dimethyl arsenic acid and dimethyl arsenate) induced vomiting, abdominal pain, hyperactive bowel, and diarrhea (Lee et al. 1995).

The gastrointestinal tract appears to be the critical target of toxicity following oral exposure to MMA. Diarrhea/loose feces has been reported in mice and rabbits following a single gavage dose of 2,200 mg MMA/kg or 60 mg MSMA/kg, respectively (Jaghabir et al. 1988; Kaise et al. 1989), pregnant rabbits administered 12 mg MMA/kg/day via gavage (Irvine et al. 2006), rats exposed to 30.2 mg MMA/kg/day in the diet during the first year of a 2-year study (Arnold et al. 2003), dogs administered 2 mg MMA/kg/day via capsule for 52 weeks (Waner and Nyska 1988), rats fed diets containing 25.7 mg MMA/kg/day for 2 years (Arnold et al. 2003), and mice exposed to 67.1 mg MMA/kg/day in the diet for

2 years (Arnold et al. 2003). However, the increased incidence of diarrhea is not always accompanied by macroscopic or histological alterations in the gastrointestinal tissues. For example, in the 2-year rat study (Arnold et al. 2003; incidence data reported in Crown et al. 1990), an increased incidence of diarrhea was observed at 25.7 mg MMA/kg/day; macroscopic or histological alterations were observed in some animals, but the incidence was similar to controls. At the next highest dose level (72.4 mg MMA/kg/day), thickened wall and edema and hemorrhagic, necrotic, ulcerated, or perforated mucosa were observed in the large intestine and significant increases in the incidence of squamous metaplasia of the epithelial columnar absorptive cells were found in the cecum, colon, and rectum. Squamous metaplasia was also observed in the cecum and colon of mice chronically exposed to 67.1 mg MMA/kg/day (Arnold et al. 2003; incidence data reported in Gur et al. 1991).

There are some reports of gastrointestinal effects in rats and dogs exposed to DMA; however, the LOAELs for these effects are higher than the LOAELs for MMA and most rodent studies do not report effects at nonlethal doses. Diarrhea with congestion and hemorrhagic gastrointestinal contents were observed in rats exposed to a lethal dose of 190 mg DMA/kg/day in the diet for 4 weeks (Crown et al. 1987) and diarrhea and vomiting were reported in dogs administered 16 mg DMA/kg/day via capsule 6 days/week (Zomber et al. 1989). No gastrointestinal effects were observed in rats or mice chronically exposed to 7.8 or 94 mg DMA/kg/day, respectively (Arnold et al. 2006).

Vomiting and gastrointestinal hemorrhage were observed in dogs after a single capsulized dose of 50 mg/kg roxarsone (Kerr et al. 1963), although slightly higher doses administered for 13 weeks to rats and mice had no effect (NTP 1989b). No gastrointestinal effects were seen after chronic exposure of rats (4 mg/kg/day) or mice (43 mg/kg/day) to roxarsone (NTP 1989b).

Hematological Effects.

Inorganic Arsenicals. Anemia and leukopenia are common effects of arsenic poisoning in humans, and have been reported following acute (Armstrong et al. 1984; Goldsmith and From 1986; Mizuta et al. 1956; Muzi et al. 2001; Westhoff et al. 1975), intermediate (Franzblau and Lilis 1989; Heyman et al. 1956; Nagai et al. 1956; Wagner et al. 1979), and chronic oral exposures (Chakraborti et al. 2003a; Glazener et al. 1968; Guha Mazumder et al. 1988; Hopenhayn et al. 2006; Kyle and Pease 1965; Tay and Seah 1975) at doses of 0.002 mg As/kg/day or more. These effects may be due to both a direct cytotoxic or hemolytic effect on the blood cells (Armstrong et al. 1984; Fincher and Koerker 1987; Goldsmith and From 1986; Kyle and Pease 1965; Lerman et al. 1980) and a suppression of erythropoiesis (Kyle and

Pease 1965; Lerman et al. 1980). However, hematological effects are not observed in all cases of arsenic exposure (EPA 1981b; Harrington et al. 1978; Huang et al. 1985; Silver and Wainman 1952) or even all acute poisoning cases (Cullen et al. 1995; Moore et al. 1994b).

In an acute animal study, Tice et al. (1997) found that there was a decrease in polychromatic erythrocytes in the bone marrow of mice treated with 6 mg As/kg/day for 1 or 4 days. There was no effect at 3 mg As/kg/day. Long-term studies found mild anemia in dogs fed arsenite or arsenate for 2 years at 2.4 mg As/kg/day, but no hematological effect in dogs fed 1 mg As/kg/day for 2 years or 1.9 mg As/kg/day for 26 weeks (Byron et al. 1967; Neiger and Osweiler 1989). Chronic rat studies found little or no evidence of anemia at doses up to 30 mg As/kg/day, even with co-exposure to lead (Byron et al. 1967; Kroes et al. 1974). No hematological effects were found in monkeys exposed to arsenic doses of 3–6 mg As/kg/day for 1 year (Heywood and Sortwell 1979).

Rats exposed to arsenate for 6 weeks had decreased activities of several enzymes involved in heme synthesis, but data were not provided on whether this resulted in anemia (Woods and Fowler 1977, 1978). Exposure of rats to ≥5 ppm of arsenic (0.30 mg As/kg/day as sodium arsenite) in the drinking water for 4 weeks resulted in increased platelet aggregation, while 10 or 25 ppm (0.60 or 1.5 mg As/kg/day) was associated with increased P-selectin-positive cells and decreased occlusion time (Lee et al. 2002), representing a change in platelet function. Similarly, exposure of rats or guinea pigs to 10 or 25 ppm of arsenic as arsenite (approximate doses of 0, 0.92, or 2.3 mg As/kg/day for rats and 0, 0.69, or 1.7 mg As/kg/day for guinea pigs) in the drinking water for 16 weeks (Kannan et al. 2001) resulted in decreases in erythrocyte and leukocyte numbers (rats and guinea pigs), increased blood mean corpuscular volume and corpuscular hemoglobin mass (guinea pigs only), and decreased mean corpuscular hemoglobin concentration (rats only). Gallium arsenide also disrupts heme synthesis in rats, although the evidence suggests that this effect is due primarily to the gallium moiety (Flora et al. 1997a).

Organic Arsenicals. No adverse hematological effects were noted in a man who ingested 78 mg/kg as dimethyl arsenic acid and dimethyl arsenate (Lee et al. 1995). No hematological effects were observed in rats exposed to 8.4 or 72.4 mg MMA/kg/day for 2 years (Arnold et al. 2003; Shen et al. 2003) or dogs administered 35 mg MMA/kg/day for 52 weeks (Waner and Nyska 1988); additionally, no alterations in total or differential leukocyte levels were observed in mice exposed to 67.1 mg MMA/kg/day for 2 years (Arnold et al. 2003). Although some studies have reported hematological alterations following oral exposure to DMA, this is not a consistent finding. Observed alterations include decreased mean corpuscular hemoglobin concentration in rats exposed to 2.3 mg DMA/kg/day for 10 weeks (Rubin et al.

1989), decreased hemoglobin and erythrocyte levels in rats exposed to 4.5 mg DMA/kg/day for 13 weeks (Crown et al. 1987), decreased erythrocyte levels and increased leukocyte levels in dogs administered capsules containing 40 mg DMA/kg/day for 52 weeks (Zomber et al. 1989), and decreased lymphocyte and increased monocyte levels were observed in mice chronically exposed to 94 mg DMA/kg/day (Arnold et al. 2006). No hematological alterations have been observed in rats exposed to 7.8 mg DMA/kg/day for 2 years (Arnold et al. 2006). Similarly, no hematological effects were observed in rats (Kerr et al. 1963; NTP 1989b), mice (NTP 1989b), or dogs (Prier et al. 1963) exposed to 20–32, 68, or 5 mg/kg/day roxarsone, respectively, for intermediate or chronic durations

Musculoskeletal Effects.

Inorganic Arsenicals. No studies were located regarding musculoskeletal effects in humans or animals after oral exposure to inorganic arsenicals.

Organic Arsenicals. No studies were located regarding musculoskeletal effects in humans after oral exposure to organic arsenicals. No musculoskeletal effects were seen after intermediate or chronic exposure of rats and mice to MMA (Arnold et al. 2003), DMA (Arnold et al. 2006), or roxarsone (NTP 1989b).

Hepatic Effects.

Inorganic Arsenicals. A number of studies in humans exposed to inorganic arsenic by the oral route have noted signs or symptoms of hepatic injury. Clinical examination often reveals that the liver is swollen and tender (Chakraborty and Saha 1987; Franklin et al. 1950; Guha Mazumder et al. 1988, 1998a; Liu et al. 2002; Mizuta et al. 1956; Silver and Wainman 1952; Wade and Frazer 1953; Zaldívar 1974), and analysis of blood sometimes shows elevated levels of hepatic enzymes (Armstrong et al. 1984; Franzblau and Lilis 1989; Guha Mazumder 2005; Hernández-Zavala et al. 1998). These effects are most often observed after repeated exposure to doses of 0.01–0.1 mg As/kg/day (Chakraborty and Saha 1987; Franklin et al. 1950; Franzblau and Lilis 1989; Guha Mazumder et al. 1988; Mizuta et al. 1956; Silver and Wainman 1952; Wade and Frazer 1953), although doses as low as 0.006 mg As/kg/day have been reported to have an effect following chronic exposure (Hernández-Zavala et al. 1998). Hepatic effects have also been reported in acute bolus poisoning cases at doses of 2 mg As/kg/day or more (Hantson et al. 1996; Kamijo et al. 1998; Levin-Scherz et al. 1987; Quatrehomme et al. 1992; Vantroyen et al. 2004), although acute exposure to 19 mg As/kg did not cause hepatic effects in an infant (Cullen et al. 1995).

Histological examination of the livers of persons chronically exposed to similar doses has revealed a consistent finding of portal tract fibrosis (Guha Mazumder 2005; Guha Mazumder et al. 1988; Morris et al. 1974; Piontek et al. 1989; Szuler et al. 1979), leading in some cases to portal hypertension and bleeding from esophageal varices (Szuler et al. 1979); cirrhosis has also been reported at an increased frequency in arsenic-exposed individuals (Tsai et al. 1999). Several researchers consider that these hepatic effects are secondary to damage to the hepatic blood vessels (Morris et al. 1974; Rosenberg 1974), but this is not directly established.

Acute exposure of monkeys to 6 mg As/kg/day resulted in vacuolization of the hepatocytes (Heywood and Sortwell 1979). Studies in dogs or mice have not detected clinically significant hepatic injury following exposure to either arsenite or arsenate (Byron et al. 1967; Fowler and Woods 1979; Kerkvliet et al. 1980; Neiger and Osweiler 1989; Schroeder and Balassa 1967), although enlargement of the common bile duct was noted in rats fed either arsenate or arsenite in the diet for 2 years (Byron et al. 1967; Kroes et al. 1974) and lipid vacuolation and fibrosis were seen in the livers of rats exposed to 12 mg As/kg/day as arsenate in the drinking water for 6 weeks (Fowler et al. 1977). Similarly, fatty changes and inflammatory cell infiltration were seen in the livers of both normal and metallothionein-null mice exposed to 5.6 mg arsenic/kg/day in the drinking water for 48 weeks (Liu et al. 2000). Increases in liver zinc and copper concentrations were noted in rats receiving a single oral dose of 10 mg As/kg as sodium arsenite (Flora and Tripathi 1998) and hepatic levels of malondialdehyde were increased and glutathione levels were decreased in livers of rats receiving 200 mg As/kg as GaAs (Flora et al. 1998). An increase in indices of peroxidation was reported in rats dosed with approximately 0.02 mg As/kg/day for 60 days from drinking water containing 2.5 mg sodium arsenite/L (Bashir et al. 2006); absolute liver weight was also increased at this dose level. Elevated levels of serum aspartate aminotransferase (AST) were observed in rats administered a single oral dose of 100 mg As/kg as GaAs (Flora et al. 1998). Exposure of guinea pigs to 0.69 or 1.7 mg As/kg/day in the drinking water for 16 weeks, but not in rats exposed to 0.92 or 2.3 mg As/kg/day, resulted in increases in delta-aminolevulinic acid synthetase (ALAS) levels (Kannan et al. 2001). Exposure of BALB/C mice to 0.7 mg arsenic/kg/day in the drinking water for 15 months resulted in increased liver weights, changes in liver enzymes (glutathione S-transferase, glutathione reductase, catalase, glucose-6-phosphate dehydrogenase, glutathione peroxidase), fatty liver, and fibrosis (Santra et al. 2000).

Organic Arsenicals. No adverse hepatic effects were noted after ingestion of 1,714 mg/kg MSMA or 78 mg DMA/kg (as dimethyl arsenic acid and dimethyl arsenate) in a suicide attempt (Lee et al. 1995; Shum et al. 1995). No other studies of the hepatic effects of organic arsenicals in humans were located.

Histological examination of livers from rabbits given repeated oral doses of MMA showed diffuse inflammation and hepatocellular degeneration (Jaghabir et al. 1989), but the lesions were not severe. Male rats exposed to a time-weighted average (TWA) dose of 72.4 mg MMA/kg/day for 104 weeks showed a decrease in absolute liver weight, while females exposed to 98.5 mg MMA/kg/day showed histiocytic proliferation of the liver (Arnold et al. 2003); however, these effects were probably due to a decrease in body weight and secondary complications of perforation and ulceration of the gastrointestinal effect, respectively. Shen et al. (2003) reported increases in and the number of GST-P-positive foci in the livers of rats exposed to average concentrations of 8.4 mg MMA/kg/day in the diet for 104 weeks. No effects were observed in rats exposed to DMA (Siewicki 1981), but mice exposed to one or two oral doses of 720 mg DMA/kg had decreased liver glutathione and cytochrome P-450 content and serum ornithine decarboxylase activity (Ahmad et al. 1999a). Generalized icterus was reported in dogs after acute exposure to roxarsone (Kerr et al. 1963). Some small fluctuations in liver weight have been noted in rats and mice after intermediate oral exposure to roxarsone, but the toxicological significance of this is not clear and is not observed after chronic exposure of rats and mice to lower doses (NTP 1989b).

Renal Effects.

Inorganic Arsenicals. Most case studies of acute and chronic arsenic toxicity do not report clinical signs of significant renal injury, even when other systems are severely impaired (e.g., Cullen et al. 1995; Franzblau and Lilis 1989; Jenkins 1966; Kersjes et al. 1987; Mizuta et al. 1956; Silver and Wainman 1952). In some cases, elevated serum levels of creatinine or bilirubin have been noted (Armstrong et al. 1984; Levin-Scherz et al. 1987; Moore et al. 1994b), and mild proteinuria may occur (Armstrong et al. 1984; Glazener et al. 1968; Tay and Seah 1975). Acute renal failure in some bolus poisoning episodes (e.g., Fincher and Koerker 1987; Goebel et al. 1990; Levin-Scherz et al. 1987; Lugo et al. 1969; Moore et al. 1994b) is probably a result of fluid imbalances or vascular injury (Rosenberg 1974; Zaldívar 1974). Glomerular congestion has been observed after an acute exposure to high doses (Quatrehomme et al. 1992).

Studies in animals also indicate that the kidney is not a major target organ for inorganic arsenic (Byron et al. 1967; Schroeder and Balassa 1967; Woods and Southern 1989), although some effects have been reported at high exposure levels. Mild histological changes in the renal tubules of monkeys exposed to arsenate for 2 weeks were noted by Heywood and Sortwell (1979), and some mild alterations in renal mitochondria in rats exposed to arsenate for 6 weeks were noted by Brown et al. (1976). Mild proteinuria

(Flora et al. 1998) and an increase in kidney zinc concentration (Flora and Tripathi 1998) have also been noted in rats exposed orally to a single dose of 100 mg As/kg as GaAs or 10 mg As/kg as sodium arsenite, respectively. These data suggest that the kidney is relatively less sensitive to arsenic than most other organ systems, and renal effects are unlikely to be of concern except secondary to fluid imbalances or cardiovascular injury.

Organic Arsenicals. No adverse renal effects were noted after ingestion of 1,714 mg MSMA/kg in a suicide attempt (Shum et al. 1995). Animal studies have reported renal and urinary bladder effects following oral exposure to organic arsenicals; the available data suggest that the urinary system is a more sensitive target for DMA, than for MMA or roxarsone. A decrease in urine volume was observed in rabbits following a single gavage dose of 30 mg MSMA/kg/day (Jaghabir et al. 1988) and a decrease in urine volume (35 mg MMA/kg/day) and an increase in urine specific gravity (8 mg MMA/kg/day) were observed in dogs administered MMA via capsule for 52 weeks (Waner and Nyska 1988). However, these effects may be indicative of dehydration due to diarrhea rather than a direct effect on the kidney. In a 2-year study in rats (Arnold et al. 2003), an increase in the severity of progressive glomerulonephropathy was observed in females at 33.9 mg MMA/kg/day. Hydronephrosis, pyelonephritis, cystitis, and decreases in urine volume and pH were also observed 72.4 mg MMA/kg/day; however, the investigators noted that these lesions probably resulted from urinary tract obstruction, which was secondary to peritonitis caused by gastrointestinal tract ulcerations. An increased incidence of progressive glomerulonephropathy was also observed in male mice exposed to ≥6.0 mg MMA/kg/day in the diet for 2 years (Arnold et al. 2003; incidence data reported in Gur et al. 1991); the investigators (Gur et al. 1991) noted that the kidney lesions were consistent with the normal spectrum of spontaneous lesions and that there were no differences in character or severity of the lesions between the different groups.

Exposure to DMA has resulted in kidney effects in rats and mice exposed to at least 3.1 or 37 mg DMA/kg/day, respectively; no renal effects were observed in dogs exposed to doses as high as 40 mg As/kg/day for 52 weeks (Zomber et al. 1989). In rats, the renal damage is characterized by increased urine volume and pH, decreased urine osmolarity and electrolyte (sodium, potassium, chlorine) levels, increased urinary calcium levels, and increased organ weight, nephrocalcinosis, and necrosis in the renal papillae and/or cortex; an increase in water consumption is also typically observed. The LOAELs for these effects are 5–57 mg DMA/kg/day in intermediate-duration studies (Arnold et al. 1999; Crown et al. 1987; Murai et al. 1993) and 3.1 mg DMA/kg/day in a chronic-duration study (Arnold et al. 2006). Another study did not find renal effects in rats exposed to 16.5 mg DMA/kg/day (Rubin et al. 1989). This study involved exposure to Sprague-Dawley rats compared to Fischer 344 rats used in the studies with

positive results; it is not known if this reflects a difference in strain sensitivity. In mice, progressive glomerulonephropathy was observed at 37 mg DMA/kg/day and nephrocalcinosis was observed at 94 mg DMA/kg/day (Arnold et al. 2006).

Increased kidney weights and minimal tubular epithelial cell degeneration, tubular casts, and focal mineralization were observed in rats exposed to 32 mg/kg/day roxarsone for 13 weeks (NTP 1989b). No adverse effects were observed in rats at doses as high as 20 mg/kg/day (Kerr et al. 1963; NTP 1989b) for 13 weeks or 10 mg/kg/day for 2 years (NTP 1989b; Prier et al. 1963). No adverse renal effects have been observed in mice exposed to roxarsone doses as high as 136 mg/kg/day (NTP 1989b) or 43 mg/kg/day (NTP 1989b; Prier et al. 1963) for intermediate or chronic durations, respectively, or in dogs exposed to 5 mg/kg/day for a chronic duration (Prier et al. 1963).

Damage to the urinary bladder has been observed in several studies in which rats were exposed to DMA. The observed effects include altered bladder cell surface characteristics in rats exposed to 11 mg DMA/kg/day in the diet for 2 weeks (Cohen et al. 2001), increased bladder weight and regenerative proliferation (measured as an increase in BrdU labeling) in bladder epithelium at 5 mg DMA/kg/day for 10 weeks (Arnold et al. 1999), necrosis of bladder epithelium at 10 mg DMA/kg/day for 10 weeks (Arnold et al. 1999), nodular or papillar hyperplasia at 3.4 mg DMA/kg/day for 2 years (Wei et al. 2002), and urothelial vacuolar degeneration and hyperplasia of urothelial cells at 3.1 mg DMA/kg/day for 2 years (Arnold et al. 2006). Vacuolization of the urothelium in the urinary bladder have also been observed in mice exposed to 7.8 mg DMA/kg/day in the diet for 2 years (Arnold et al. 2006). Inconsistent results were found for MMA. Hyperplasia was observed in the bladders of rats exposed to 1 mg As/kg/day as MMA in drinking water for 2 years (Shen et al. 2003), but bladder effects were not observed in another 2-year study (Arnold et al. 2003) in which rats were exposed to doses as high as 34.8 mg As/kg/day as MMA in the diet. No urinary bladder effects were found in rats and mice exposed to 64 or 136 mg/kg/day roxarsone for 13 weeks (NTP 1989b) or 4 or 43 mg/kg/day roxarsone for 2 years (NTP 1989b).

Endocrine Effects.

Inorganic Arsenicals. Very little has been written about the effects of oral exposure to arsenic on endocrine glands. In a report of the autopsies of five children who died in Chile after chronic exposure to arsenic in the drinking water, arterial thickening in the pancreas was noted (Rosenberg 1974). An association has been demonstrated between exposure to arsenic in drinking water and an increased

incidence of diabetes mellitus (Lai et al. 1994; Rahman et al. 1998; Tsai et al. 1999; Tseng et al. 2000; Wang et al. 2003), although dose-response relationships are not available.

Exposure of rats to 2.3 mg As/kg/day as arsenic trioxide for 30 days resulted in reductions in the number of islet cells in the pancreas, as well as significant reductions in pancreatic superoxide dismutase (SOD) and catalase enzyme levels and increases in the production of nitric oxide and malondialdehyde (Mukherjee et al. 2004).

Organic Arsenicals. No studies of effects of organic arsenic compounds on endocrine glands in humans were found. Hypertrophy of thyroid epithelium was observed in rats exposed to 33.9 mg MMA/kg/day in the diet for 2 years (Arnold et al. 2003), 4.0 mg DMA/kg/day in the diet for 13 weeks (Crown et al. 1987), 16.5 mg DMA/kg/day in the diet for at least 10 weeks (Rubin et al. 1989), and 7.8 mg DMA/kg/day in the diet for 2 years (Arnold et al. 2006). No other biologically significant effects were observed in other endocrine tissues following exposure to MMA or DMA. No adverse effects were seen in the adrenal or pituitary glands, thyroid, or pancreas after intermediate or chronic exposure of rats (20–64 or 4 mg/kg/day, respectively) and mice (136 or 43 mg/kg/day, respectively) to roxarsone (NTP 1989b).

Dermal Effects.

Inorganic Arsenicals. One of the most common and characteristic effects of arsenic ingestion is a pattern of skin changes that include generalized hyperkeratosis and formation of hyperkeratotic warts or corns on the palms and soles, along with areas of hyperpigmentation interspersed with small areas of hypopigmentation on the face, neck, and back. These and other dermal effects have been noted in a large majority of human studies involving repeated oral exposure (e.g., Ahmad et al. 1997, 1999b; Ahsan et al. 2000; Bickley and Papa 1989; Borgoño and Greiber 1972; Borgoño et al. 1980; Cebrián et al. 1983; Chakraborti et al. 2003a, 2003b; Chakraborty and Saha 1987; Foy et al. 1992; Franklin et al. 1950; Franzblau and Lilis 1989; Guha Mazumder et al. 1988, 1998a, 1998b, 1998c; Guo et al. 2001a; Haupert et al. 1996; Huang et al. 1985; Lander et al. 1975; Liu et al. 2002; Lüchtrath 1983; Milton et al. 2004; Mizuta et al. 1956; Morris et al. 1974; Nagai et al. 1956; Piontek et al. 1989; Rosenberg 1974; Saha and Poddar 1986; Silver and Wainman 1952; Szuler et al. 1979; Tay and Seah 1975; Tseng et al. 1968; Wade and Frazer 1953; Wagner et al. 1979; Wong et al. 1998a, 1998b; Zaldívar 1974, 1977). In cases of low-level chronic exposure (usually from water), these skin lesions appear to be the most sensitive indication of effect, so this end point is considered to be the most appropriate basis for establishing a chronic oral MRL. This is supported by the finding that other effects (hepatic injury, vascular disease, neurological

effects) also appear to have similar thresholds. As shown in Table 3-3 and Figure 3-3, numerous studies in humans have reported dermal effects at chronic dose levels generally ranging from about 0.01 to 0.1 mg As/kg/day (Ahmad et al. 1997; Bickley and Papa 1989; Borgoño and Greiber 1972; Borgoño et al. 1980; Cebrián et al. 1983; Chakraborty and Saha 1987; Foy et al. 1992; Franklin et al. 1950; Guha Mazumder et al. 1988; Huang et al. 1985; Lüchtrath 1983; Piontek et al. 1989; Silver and Wainman 1952; Tseng et al. 1968; Zaldívar 1974, 1977). However, in a study with detailed exposure assessment, all confirmed cases of skin lesions ingested water containing >100 μg/L arsenic (approximately 0.0037 mg As/kg/day) and the lowest known peak arsenic concentration ingested by a case was 0.115 μg/L (approximately 0.0043 mg As/kg/day) (Haque et al. 2003). Another large study reported increased incidence of skin lesions associated with estimated doses of 0.0012 mg As/kg/day (0.023 mg As/L drinking water) (Ahsan et al. 2006). Several epidemiological studies of moderately sized populations (20–200 people) exposed to arsenic through drinking water have detected no dermal or other effects at average chronic doses of 0.0004–0.01 mg As/kg/day (Cebrián et al. 1983; EPA 1981b; Guha Mazumder et al. 1988; Harrington et al. 1978; Valentine et al. 1985), and one very large study detected no effects in any person at an average total daily intake (from water plus food) of 0.0008 mg As/kg/day (Tseng et al. 1968). This value has been used to calculate a chronic oral MRL for inorganic arsenic of 0.0003 mg/kg/day, as described in footnote c in Table 3-3.

Another prominent dermal effect associated with chronic ingestion of inorganic arsenic is skin cancer. As discussed in greater detail in Section 3.2.2.7 (below), some of these skin cancers may evolve from the hyperkeratotic corns or warts, while the areas of altered pigmentation are not considered to be precancerous (EPA 1988d).

Dermal lesions similar to those observed in humans have not been noted in oral exposure studies in monkeys (Heywood and Sortwell 1979), dogs (Byron et al. 1967), or rodents (Schroeder et al. 1968). However, a hyperplastic response to oral arsenic exposure was reported in arsenic-exposed mice (Rossman et al. 2004).

Organic Arsenicals. No studies were located regarding dermal effects in humans after oral exposure to organic arsenicals. No gross or histological skin alterations were observed in rats or mice following intermediate- or chronic-duration exposure to MMA (Arnold et al. 2003; as reported in Crown et al. 1990; Gur et al. 1991), DMA (Arnold et al. 2006; as reported in Gur et al. 1989a, 1989b), or roxarsone (NTP 1989b)

Ocular Effects.

Inorganic Arsenicals. Periorbital swelling was reported in people drinking contaminated well water at an approximate dose of 0.2 mg As/kg for 1 week (Armstrong et al. 1984). Facial edema, generally involving the eyelids, was a prominent feature of arsenic poisoning among 220 cases associated with an episode of arsenic contamination of soy sauce in Japan (Mizuta et al. 1956). Exposure was to an estimated dose of 0.05 mg/kg/day and lasted for up to 2–3 weeks. The edema developed soon after the initial exposure and then subsided. This effect forms the basis (in part) for the acute oral MRL of 0.005 mg/kg/day for inorganic arsenic, as described in footnote b in Table 3-3. Nemec et al. (1998) noted the appearance of dried red material around the eyes of mice receiving daily oral doses of 24 mg As/kg as arsenic acid for 10 days during gestation.

Organic Arsenicals. No studies were located regarding ocular effects in humans or animals after oral exposure to organic arsenicals. No gross or histological alterations in the eye were observed in rats or mice following intermediate- or chronic-duration exposure to MMA (Arnold et al. 2003; as reported in Crown et al. 1990; Gur et al. 1991), DMA (Arnold et al. 2006; as reported in Gur et al. 1989a, 1989b), or roxarsone (NTP 1989b).

Body Weight Effects.

Inorganic Arsenicals. A 41-year old woman exposed to arsenic in the drinking water for 4 months at an approximate dose of 0.06 mg As/kg/day reported losing 40 pounds (18 kg) of body weight before seeking treatment (Wagner et al. 1979). Weight loss was also among the effects observed in a series of 475 chronic arsenism patients hospitalized in Antofagasto, Chile after receiving approximate doses of 0.02 mg As/kg/day in the drinking water for an unspecified number of years (Zaldívar 1974).

Reductions in body weight gain are commonly seen in animal studies of ingested arsenic. In pregnant rats, body weight gain was reduced by gavage treatment with 23 mg As/kg/day as arsenic trioxide on day 9 of gestation (NOAEL=15 mg As/kg/day, Stump et al. 1999), and by repeated gavage treatment with 8 mg As/kg/day as arsenic trioxide from 2 weeks prior to mating through gestation (NOAEL=4 mg As/kg/day, Holson et al. 2000). Exposure of rats by gavage to 26.6 mg As/kg/day as sodium arsenite, but not 13.3 mg As/kg/day or lower, 5 days/week for 4 weeks resulted in a significant decrease in body weight (Schulz et al. 2002). In 6-week rat studies, body weight gain was decreased at 11–12 mg As/kg/day, but not at 6–9 mg As/kg/day (Brown et al. 1976; Fowler et al. 1977). In a 12-week oral

gavage study, rats dosed with 1.5 mg/kg/day sodium arsenite had a median final body weight 18% lower than controls Dhar et al. (2005). A 60-day rat study with sodium arsenite in the drinking water reported a 13% reduction in final body weight in rats dosed with approximately 0.02 mg As/kg/day (Bashir et al. 2006). In chronic rat studies of arsenate and arsenite, body growth decreases were found at doses as low as 2 mg As/kg/day in feeding studies (Byron et al. 1967; Kroes et al. 1974), while rats exposed to lower levels of sodium arsenite in the drinking water (0.6 mg As/kg/day) throughout their lifetimes grew normally (Schroeder et al. 1968). Rats given a single oral dose of 100 mg As/kg as GaAs exhibited a 15% reduction in body weight compared to controls 7 days after exposure (Flora et al. 1998). Body weight gain was decreased in mice at 24 mg As/kg/day in a gestation exposure study (Nemec et al. 1998), 10 mg As/kg/day in a 6-week study (Fowler and Woods 1979), and 1 mg As/kg/day in a 2-year study (Schroeder and Balassa 1967). Growth was unaffected in mice that received 12 mg As/kg/day in the gestation exposure study (Nemec et al. 1998), 5 mg As/kg/day in the 6-week study (Fowler and Woods 1979), or 0.7–0.8 mg As/kg/day in 1–3 month arsenate drinking water studies (Healy et al. 1998). Dogs chronically treated with 2.4 mg As/kg/day as sodium arsenite lost 44–61% of their starting body weight and died, while lower doses had no effect on growth (Byron et al. 1967). Weight depression was also reported in dogs chronically treated with 2.4 mg As/kg/day as sodium arsenate (Byron et al. 1967). Feed consumption and body weight gain were significantly reduced in a dose-related manner in dogs fed 1.5 or 1.9 mg As/kg/day as sodium arsenite in the diet (Neiger and Osweiler 1989). Dogs in the high-dose group lost 25% of their body weight over the 17-week study period. Pair-fed controls lost weight at the same rate as high-dose dogs, showing that the effect on body weight was due to reduced feed consumption, rather than a direct effect of arsenic.

Organic Arsenicals. No studies were located regarding body weight effects in humans after oral exposure to organic arsenicals. In animal studies of organic arsenicals, decreases in body weight gain were observed in rats and mice after acute, intermediate, and chronic duration exposure to MMA (Arnold et al. 2003; Waner and Nyska 1988), DMA (Murai et al. 1993), and roxarsone (NTP 1989b); decreases in body weight gain have also been reported in pregnant rats and rabbits exposed to MMA (Irvine et al. 2006) or DMA (Irvine et al. 2006; Rogers et al. 1981). For MMA, the decreases in body weight gain were observed following intermediate-duration exposure of rats and dogs to 106.9 or 8 mg MMA/kg/day (Arnold et al. 2003; Waner and Nyska 1988), respectively, and following chronic-duration exposure of rats, mice, and dogs to 25.7, 67.1, or 8 mg MMA/kg/day, respectively (Arnold et al. 2003; Waner and Nyska 1988). The decreases in body weight gain occurred at doses that were associated with diarrhea and histological alterations in the gastrointestinal tract (Arnold et al. 2003; Waner and Nyska 1988). One DMA study in nonpregnant animals reported decreases in body weight gain in rats administered 57 mg

DMA/kg/day via gavage 5 days/week for 4 weeks (Murai et al. 1993); other DMA studies have not reported decreases in body weight gain in rats following exposure to 11 mg DMA/kg/day for acute durations (Cohen et al. 2001), 3.7–60 mg DMA/kg/day for intermediate durations (Arnold et al. 1999; Crown et al. 1987; Rubin et al. 1989; Siewicki 1981; Wanibuchi et al. 1996; Yamamoto et al. 1995), or 0.77 mg DMA/kg/day for chronic durations (Arnold et al. 2006). No alterations in body weight gain were observed in mice exposed to 94 mg DMA/kg/day for 2 years (Arnold et al. 2006). The lowest doses of roxarsone to produce a decrease in growth were 32 and 16 mg/kg/day in rats following acute- or intermediate-duration exposure, respectively, and 168 and 136 mg/kg/day in mice following acute or intermediate exposure (NTP 1989b); at the highest dose tested in chronic studies, no significant alterations in body weight gain were observed in rats at 4 mg/kg/day or in mice at 43 mg/kg/day (NTP 1989b).

3.2.2.3 Immunological and Lymphoreticular Effects

Inorganic Arsenicals. No studies were located regarding immunological and lymphoreticular effects in humans after oral exposure to inorganic arsenicals. No evidence of immunosuppression was detected in mice exposed to arsenate at levels up to 100 ppm (20 mg As/kg/day) in drinking water (Kerkvliet et al. 1980). This NOAEL is shown in Table 3-3 and Figure 3-3. Gallium arsenide at doses of 52–260 mg As/kg/day produced significant, dose-related decreases in relative spleen weight, spleen cellularity, humoral immune response (antibody forming cell response to sheep RBC), and delayed type hypersensitivity in rats (Flora et al. 1998). However, it is not clear to what extent these effects are due to the arsenic moiety.

Organic Arsenicals. No studies were located regarding immunological and lymphoreticular effects in humans or animals after oral exposure to organic arsenicals. No histological alterations were observed in immunological or lymphoreticular tissues following intermediate-duration exposure of rats to 43.2 mg DMA/kg/day in the diet (Crown et al. 1987) or rats and mice to 18.23 or 38.7 mg As/kg/day as roxarsone, respectively (NTP 1989b) or following chronic-duration exposure of rats and mice to 72.4 or 67.1 mg MMA/kg/day (Arnold et al. 2003), 7.8 or 94 mg DMA/kg/day (Arnold et al. 2006), or 4 or 43 mg/kg/day roxarsone (NTP 1989b). No studies examined immune function following oral exposure to organic arsenicals.

3.2.2.4 Neurological Effects

Inorganic Arsenicals. A large number of epidemiological studies and case reports indicate that ingestion of inorganic arsenic can cause injury to the nervous system. Acute, high-dose exposures (2 mg As/kg/day or above) often lead to encephalopathy, with signs and symptoms such as headache, lethargy, mental confusion, hallucination, seizures, and coma (Armstrong et al. 1984; Bartolome et al. 1999; Civantos et al. 1995; Cullen et al. 1995; Danan et al. 1984; Fincher and Koerker 1987; Levin-Scherz et al. 1987; Quatrehomme et al. 1992; Uede and Furukawa 2003; Vantroyen et al. 2004). Repeated exposures to lower levels (0.03–0.1 mg As/kg/day) are typically characterized by a symmetrical peripheral neuropathy (Chakraborti et al. 2003a, 2003b; Foy et al. 1992; Franzblau and Lilis 1989; Guha Mazumder et al. 1988; Hindmarsh et al. 1977; Huang et al. 1985; Lewis et al. 1999; Mizuta et al. 1956; Muzi et al. 2001; Silver and Wainman 1952; Szuler et al. 1979; Wagner et al. 1979). This neuropathy usually begins as numbness in the hands and feet, but later may develop into a painful "pins and needles" sensation. Both sensory and motor nerves are affected, and muscle weakness often develops, sometimes leading to wrist-drop or ankle-drop (Chhuttani et al. 1967; Heyman et al. 1956). Diminished sensitivity to stimulation and abnormal patellar reflexes have also been reported (Mizuta et al. 1956). Histological examination of nerves from affected individuals reveals a dying-back axonopathy with demyelination (Goebel et al. 1990; Hindmarsh and McCurdy 1986). Some recovery may occur following cessation of exposure, but this is a slow process and recovery is usually incomplete (Fincher and Koerker 1987; Le Quesne and McLeod 1977; Murphy et al. 1981). Peripheral neuropathy is also sometimes seen following acute high-dose exposures, with or without the previously described encephalopathy (Armstrong et al. 1984; Baker et al. 2005; Fincher and Koerker 1987; Goebel et al. 1990; Hantson et al. 1996; Kamijo et al. 1998). Neurological effects were not generally found in populations chronically exposed to doses of 0.006 mg As/kg/day or less (EPA 1981b; Harrington et al. 1978; Hindmarsh et al. 1977), although fatigue, headache, dizziness, insomnia, nightmare, and numbness of the extremities were among the symptoms reported at 0.005, but not 0.004 mg As/kg/day in a study of 31,141 inhabitants of 77 villages in Xinjiang, China (Lianfang and Jianzhong 1994), and depression was reported in some Wisconsin residents exposed to 2–10 μg As/L in the drinking water for 20 years or longer (Zierold et al. 2004).

There is emerging evidence suggesting that exposure to arsenic may be associated with intellectual deficits in children. For example, Wasserman et al. (2004) conducted a cross-sectional evaluation of intellectual function in 201 children 10 years of age whose parents were part of a larger cohort in Bangladesh. Intellectual function was measured using tests drawn from the Wechsler Intelligence Scale for Children; results were assessed by summing related items into Verbal, Performance, and Full-Scale

raw scores. The mean arsenic concentration in the water was 0.118 mg/L. The children were divided into four exposure groups, representing <5.5, 5.6–50, 50–176, or 177–790 μg As/L drinking water. After adjustment for confounding factors, a dose-related inverse effect of arsenic exposure was seen on both Performance and Full-Scale subset scores; for both end points, exposure to ≥50 μg/L resulted in statistically significant differences ($p<0.05$) relative to the lowest exposure group (<5.5 μg/L). In a later report, the same group of investigators examined 301 6-year-old children from the same area (Wasserman et al. 2007). In this case, the children were categorized into the following quartiles based on water arsenic concentration: 0.1–20.9, 21–77.9, 78–184.9, and 185–864 μg/L. After adjustment for water Mn, blood lead, and sociodemographic features known to contribute to intellectual function, water arsenic was significantly negatively associated with both Performance and Processing speed raw scores. Analyses of the dose-response showed that compared to the first quartile, those in the second and third categories had significantly lower Performance raw scores ($p<0.03$ and $p=0.05$, respectively). Those in the fourth category had marginally significantly lower Full-Scale and Processing Speed raw scores. It should be mentioned, however, that in general, arsenic in the water explained <1% of the variance in test scores. Water arsenic made no contribution to IQ outcomes. A study of 351 children age 5–15 years from West Bengal, India, found significant associations between urinary arsenic concentrations and reductions in scores of tests of vocabulary, object assembly, and picture completion; the magnitude of the reductions varied between 12 and 21% (von Ehrenstein et al. 2007). In this cohort, the average lifetime peak arsenic concentration in well water was 0.147 mg/L. However, no clear pattern was found for increasing categories of peak arsenic water concentrations since birth and children's scores in the various neurobehavioral tests conducted. Furthermore, using peak arsenic as a continuous variable in the regression models also did not support an adverse effect on the tests results. Exposure to arsenic *in utero* also did not suggest an association with the tests scores. Von Ehrestein et al. (2007) concluded that the study provided little evidence for an effect of long-term arsenic concentrations in drinking water and that the lack of findings with past exposures via drinking water may be due to incomplete assessment of past exposure, particularly exposure originating from food. Wasserman's results are consistent with those of ecological studies in children in Taiwan (Tsai et al. 2003) and in China (Wang et al. 2007). In the former, adolescents exposed to low (0.0017–0.0018 mg As/kg/day; n=20) levels of inorganic arsenic in the drinking water showed decreased performance in the switching attention task, while children in the high exposure group (0.0034–0.0042 mg As/kg/day; n=29) showed decreased performance in both the switching attention task and in tests of pattern memory, relative to unexposed controls (n=60). In the study in China (age 8– 12 years), 87 children whose mean arsenic concentration in the drinking water was 0.190 mg/L had a mean IQ score of 95 compared with 101 for children (n=253) with 0.142 mg/L arsenic in the water and 105 for control children (n=196) with 0.002 mg/L arsenic in the drinking water (Wang et

al. 2007). The differences in IQ scores between the two exposure groups and the control group were statistically significant.

Neurological effects have also been observed in animal studies. Rodriguez et al. (2001) evaluated neurobehavioral changes in male Sprague-Dawley rats exposed to 0, 5, 10, or 20 mg As/kg/day as sodium arsenite by gavage for 2 or 4 weeks; significant effects were seen in spontaneous locomotor activity and the food pellet manipulation test in the high-dose animals, while no effects were seen in the low- or mid-dose rats. Decreased performance in open field tests were also seen in rats exposed to 26.6 mg As/kg/day, but not to 13.3 mg/kg/day or less, as sodium arsenite for 4 weeks (Schulz et al. 2002); curiously, the behavioral changes were no longer present at 8 and 12 weeks of exposure, which may suggest an adaptive response. Heywood and Sortwell (1979) reported salivation and uncontrolled head shaking in two monkeys given several doses of 6 mg As/kg/day as arsenate, while no such effects were noted in monkeys given 3 mg As/kg/day for 2 weeks. Nemec et al. (1998) observed ataxia and prostration in pregnant female rabbits treated with 1.5 mg As/kg/day repeatedly during gestation, but not in rabbits treated with 0.4 mg As/kg/day. Some changes in levels of neurotransmitters (dopamine, norepinephrine, and 5-hydroxytryptamine) were seen in rats exposed to 2.3 mg As/kg/day as sodium arsenite and guinea pigs exposed to 1.7 mg As/kg/day as sodium arsenite in the drinking water for 16 weeks (Kannan et al. 2001) or in rats exposed to 0.14 mg As/kg/day as sodium arsenite by gavage for 28 days (Chattopadhyay et al. 2001), but the functional significance of these changes is not clear.

The highest NOAEL values and all reliable LOAEL values for neurological effects from inorganic arsenic in each species and duration category are recorded in Table 3-3 and plotted in Figure 3-3.

Organic Arsenicals. Numbness and tingling of the fingertips, toes, and circumoral region were reported by a women exposed to an unspecified amount of organic arsenic in bird's nest soup. Discontinuation of exposure resulted in the disappearance of symptoms (Luong and Nguyen 1999). Decreased absolute brain weights were seen in male rats exposed to 25.7 mg MMA/kg/day and female rats exposed to ≥33.9 mg MMA/kg/day, but decreased body weight also occurred at these exposure levels, and relative brain weights were increased in the males at 25.7 mg MMA/kg/day and the females at ≥33.9 mg MMA/kg/day in this study (Arnold et al. 2003). No neurological clinical signs or brain lesions were observed following chronic exposure of rats to 72.4 mg MMA/kg/day or mice to 67.1 mg MMA/kg/day (Arnold et al. 2003). Decreased spontaneous motility, increased startle response, and ataxia were observed in mice receiving a single gavage dose of 1,757 mg DMA/kg/day (Kaise et al. 1989); no other evidence (clinical signs or histological alterations) were observed in chronic studies of DMA in which

rats and mice were exposed to 7.8 or 94 mg DMA/kg/day, respectively (Arnold et al. 2006). Two studies in pigs indicate that repeated oral doses of roxarsone (6.3–20 mg/kg/day for 1 month) can cause significant neurotoxicity (Edmonds and Baker 1986; Kennedy et al. 1986; Rice et al. 1985). The main signs were time-dependent degenerations of myelin and axons (Kennedy et al. 1986; Rice et al. 1985). Evidence of neurological effects (hyperexcitability, ataxia, trembling) was noted in some rat and mouse studies (Kerr et al. 1963; NTP 1989b). Reliable NOAELs and LOAELs are presented in Tables 3-4, 3-5, and 3-6, and Figures 3-4, 3-5, and 3-6.

3.2.2.5 Reproductive Effects

Inorganic Arsenicals. Exposure to arsenic in drinking water has been associated with adverse reproductive outcomes in some studies. For example, a study of 96 women in Bangladesh who had been drinking water containing ≥0.10 mg As/L (approximately 0.008 mg As/kg/day) for 5–10 years reported a significant increase in spontaneous abortions (p=0.008), stillbirth (p=0.046), and preterm birth (p=0.018) compared to a nonexposed group (Ahmad et al. 2001). Similar results were reported by Milton et al. (2005) who found a significant association between concentrations of arsenic in the water >0.05 mg/L (approximately 0.006 mg As/kg/day) and spontaneous abortion (odds ratio [OR]=2.5; 95% CI=1.5–4.3) in a study of 533 women, also from Bangladesh. A study of 202 women from West Bengal, India, reported that exposure to arsenic concentrations of arsenic ≥0.2 mg/L in drinking water (approximately 0.02 mg As/kg/day) during pregnancy were associated with a 6-fold increased risk of stillbirth (OR=6.1; 95% CI=1.54–24.0) after adjustment for confounders (von Ehrenstein et al. 2006). No association was found between arsenic exposure and risk of spontaneous abortion (OR=1.01; 95% CI=0.73–10.8). An earlier study of 286 women in the United States also found no significant association between arsenic in the drinking water (0.0016 mg/L; approximately 0.00005 mg As/kg/day) and spontaneous abortion (OR=1.7; 95% CI=0.7–4.2) (Aschengrau et al. 1989).

Lugo et al. (1969) reported a case of a 17-year-old mother who ingested inorganic arsenic (Cowley's Rat and Mouse Poison) at week 30 of pregnancy. Twenty-four hours after ingestion of approximately 30 mL of arsenic trioxide (0.39 mg As/kg), she was admitted for treatment of acute renal failure. She went into labor and delivered a live female infant weighing 2 pounds, 7 ounces with a 1-minute Apgar score of 4. The infant's clinical condition deteriorated and she died at 11 hours of age.

Reproductive performance was not affected in female rats that received gavage doses of 8 mg As/kg/day (as As_2O_3) from 14 days prior to mating through gestation day 19 (Holson et al. 2000). Reproductive

indices that were evaluated included the precoital interval (time to mating), mating index (percentage of rats mated), and fertility index (percentage of matings resulting in pregnancy). In a 3-generation study in mice given sodium arsenite in drinking water at an average dose of 1 mg As/kg/day, there was a significant increase in the incidence of small litters and a trend toward a decreased number of pups per litter in all three generations of the treated group (Schroeder and Mitchener 1971). This finding is consistent with the results of developmental toxicity studies reported in Section 3.2.2.6. Female rats exposed to 0.24 mg As/kg/day (as arsenite) for 28 days showed changes in several reproductive system end points, including decreases in wet weights of the ovary and uterus, inhibition of steroidogenic enzymes, decreased ovarian and uterine peroxidase activities, and decreased estradiol levels relative to controls (Chattopadhyay et al. 2001). NOAEL and LOAEL values from these studies are shown in Table 3-3 and Figure 3-3.

Organic Arsenicals. No studies were located regarding reproductive effects in humans after oral exposure to organic arsenicals. No histological alterations in male or female reproductive tissues were observed in laboratory animals following exposure to MMA (Arnold et al. 2003), DMA (Arnold et al. 2006), or roxarsone (NTP 1989b) and no alterations in sperm parameters were observed in male rats exposed to 76 mg MMA/kg/day for at least 14 weeks (Schroeder 1994). However, some functional alterations have been reported in animals exposed to MMA or DMA. A decrease in estrus was observed in dogs exposed to 35 mg MMA/kg/day for 52 weeks (Waner and Nyska 1988); decreases in body weight gain (terminal body weight was 59% lower than controls) were also observed at this dose level and the effect may have been secondary to systemic toxicity. Decreases in pregnancy rate and male fertility index were observed in F_0 and F_1 rats exposed to 76 mg MMA/kg/day for 14 weeks prior to mating and during the mating, gestation, and lactation periods (Schroeder 1994). In the F_0 animals, the pregnancy rate and male fertility index were not statistically different from controls; however, the values were below historical controls and the investigators considered the effect to be treatment-related. In the F_1 animals, the male fertility index was statistically different from controls but the pregnancy rate was not; both parameters were within the range found in historical controls, but the investigators considered the effect to be treatment-related due to the consistency of the findings in the F_0 and F_1 animals. Impaired fertility, as evidenced by a decreased number of litters, was observed in male mice dosed with MSMA (119 mg/kg/day) during a 19-day mating period with unexposed females (Prukop and Savage 1986); the poor reporting of the study protocol and results precludes drawing conclusions from this study. An increase in the number of does with aborted fetuses was observed in rabbits exposed to 48 mg DMA/kg/day as DMA (Irvine et al. 2006); severe maternal toxicity (weight loss, reduced food intake, and

diarrhea) was also observed at this dose level. No reproductive effects were observed in a 2-generation rat study in which rats were exposed to 16.5 mg DMA/kg/day (Rubin et al. 1989).

3.2.2.6 Developmental Effects

Inorganic Arsenicals. Whether ingestion of inorganic arsenic may cause developmental effects in humans has not been extensively investigated. Lugo et al. (1969) reported a case of a mother who ingested inorganic arsenic (Cowley's Rat and Mouse Poison) at 30 weeks of gestation. Twenty-four hours after ingestion, she went into labor and delivered a live female infant weighing 2 pounds, 7 ounces with a 1-minute Apgar score of 4. The infant's clinical condition deteriorated with frequent episodes of apnea and bradycardia; subsequent venous blood gas determinations documented hypoxia, hypercapnea, and acidosis. The infant died at 11 hours of age. Autopsy performed 8 hours after death showed organ immaturity, generalized petechial hemorrhages, and hyaline membrane disease. Severe intra-alveolar pulmonary hemorrhage was remarkable. High arsenic levels were found in the infant’s liver, kidney, and brain, demonstrating easy passage of inorganic arsenic across the placenta. The authors considered most of the findings in the neonate to be attributable to immaturity, but suggested that arsenic may have played a role in the severe intra-alveolar hemorrhaging that contributed to death.

Chronic exposure of women to arsenic in the drinking water has been associated with infants with low birth weights in Taiwan (Yang et al. 2003) and Chile (Hopenhayn et al. 2003a). Similar associations have been made between late fetal mortality, neonatal mortality, and postneonatal mortality and exposure to high levels of arsenic in the drinking water (up to 0.86 mg/L during over a decade), based on comparisons between subjects in low- and high-arsenic areas of Chile (Hopenhayn-Rich et al. 2000). More recently, von Ehrenstein et al. (2006) reported no significant association between exposure to concentrations of ≥0.1 mg/L arsenic in drinking water (approximately 0.008 mg As/kg/day) (n=117; 29 women were exposed to ≥0.5 mg/L) and increased risk for neonatal death or infant mortality during the first year of life in a study of a population in West Bengal, India. The same group of investigators reported significantly increased SMRs for lung cancer and bronchiectasis among subjects in a city in Chile who had probable exposure *in utero* (maternal exposure) or during childhood to high levels of arsenic (near 0.9 mg/L) in the drinking water (Smith et al. 2006). For those exposed in early childhood, the SMR for lung cancer was 7.0 (95% CI=5.4–8.9, $p<0.001$) and for bronchiecstasis 12.4 (95% CI=3.3–31.7, $p<0.001$). For those born during the high-exposure period, the corresponding SMRs were 6.1 (95% CI=3.5–9.9, $p<0.001$) and 46.2 (95% CI=21.1–87.7, $p<0.001$). The mortality data analyzed were for the age range 30–49 years.

No overall association between arsenic in drinking water and congenital heart defects was detected in a case-control study in Boston (Zierler et al. 1988), although an association with one specific lesion (coarctation of the aorta) was noted (OR=3.4, 95% CI=1.3–8.9). A study of 184 women with neural tube defects in the offspring living in a Texas county bordering Mexico found that exposure to levels of arsenic in the drinking water >0.010 mg/L (range or upper limit not specified) did not significantly increase the risk for neural tube defects (OR=2.0, 95% CI=0.1–3.1) (Brender et al. 2006).

Studies in animals, however, suggest that ingested inorganic arsenic may produce developmental effects at high doses that also produce overt maternal toxicity. Rats treated with a single gavage dose of 23 mg As/kg as arsenic trioxide on day 9 of gestation had a significant increase in postimplantation loss and a decrease in viable fetuses per litter, while those treated with 15 mg As/kg showed no effects (Stump et al. 1999). Rats treated by daily gavage with 8 mg As/kg/day starting 14 days before mating and continuing through gestation had significantly reduced fetal body weights and significantly increased incidences of several skeletal variations (unossified sternebrae #5 or #6, slight or moderate sternebrae malalignment, 7th cervical ribs) that the researchers considered to be consequences of developmental growth retardation (Holson et al. 2000). No developmental effects were found at 4 mg As/kg/day in this study. Exposure of rats to 2.93–4.20 mg As/kg/day throughout gestation and for 4 months postnatally resulted in alterations in neurobehavioral parameters in the offspring, including increased spontaneous locomotor activity and number of errors in a delayed alternation task; maternal behavior was not affected (Rodriguez et al. 2002). Studies in mice found increased fetal mortality, decreased fetal body weight, a low incidence of gross malformations (primarily exencephaly), and an increase in skeletal malformations in mice given single gavage doses of 23–48 mg As/kg during gestation (Baxley et al. 1981; Hood et al. 1978), with no effects at 11 mg As/kg. Similarly, in mice treated with 24 mg As/kg/day as arsenic acid on days 6–15 of gestation, there was a significant increase in the number of resorptions per litter (42% vs. 4% in controls) and significant decreases in the number of live pups per litter (6.6 vs. 12.3 in controls) and mean fetal weight (1.0 g vs. 1.3 g in controls), while no developmental effects were found at 12 mg As/kg/day (Nemec et al. 1998). Hamsters treated with a single gavage dose of 14 mg As/kg during gestation also had increased fetal mortality and decreased fetal body weight (Hood and Harrison 1982), with no effect at 11 mg As/kg. However, the most sensitive species was the rabbit, which had increased resorptions and decreased viable fetuses per litter at 1.5 mg As/kg/day and a developmental NOAEL of 0.4 mg As/kg/day, following repeated gavage dosing with arsenic acid during gestation (Nemec et al. 1998). In each of these studies (except Hood et al. 1978, which failed to report maternal effects), overt maternal toxicity, including death in some cases, was found at the same or lower doses as the developmental

effects (Baxley et al. 1981; Holson et al. 2000; Hood and Harrison 1982; Nemec et al. 1998; Stump et al. 1999).

It is noteworthy that the effect in the 3-generation reproduction study in mice by Schroeder and Mitchener (1971), decreased pups per litter (all generations), is consistent with the findings of many of these shorter-term studies (Baxley et al. 1981; Hood and Harrison 1982; Hood et al. 1978; Nemec et al. 1998; Stump et al. 1999). The dose in this long-term study was 1 mg As/kg/day; in a 2-year study by these researchers, this dose produced effects such as decreased body weight gain and increased mortality (Schroeder and Balassa 1967).

A series of studies presented evidence that inorganic arsenic may be a transplacental carcinogen in animals. Waalkes et al. (2003, 2004a, 2004b, 2004c) exposed timed-pregnant AJ mice to 0, 42.5, or 85 ppm of sodium arsenite in the drinking water from gestation day 8 through 18 and observed the offspring for 90 weeks following birth; the study authors estimated daily doses at 9.55 and 19.3 mg As/kg/day. A dose-related increase was reported in the incidence of hepatocellular carcinomas and adrenal tumors in the male offspring from both treatment levels, while male offspring from high-dose animals showed an increase in total number of tumors. In female offspring, an increase in uterine hyperplasia was seen in the offspring of both treated groups while the offspring of high-dose animals showed increased incidence of lung carcinomas. For both exposed groups, regardless of gender, the offspring showed a significant increase in the number of malignant tumors (Waalkes et al. 2003). More recent studies from the same group of investigators have suggested that aberrant estrogen signaling, potentially through inappropriate estrogen receptor-α (ER-α), may play a role in arsenic-induced liver tumors in male offspring (Waalkes et al. 2006a) and in arsenic-induced uterine and bladder carcinoma in female offspring (Waalkes et al. 2006b). The latter was based on the observation of over-expression of ER-α and *pS*2, an estrogen-regulated gene, in the respective tissues.

These studies (shown in Table 3-3 and Figure 3-3) indicate that the fetus may be affected by ingested arsenic.

Organic Arsenicals. No studies were located regarding developmental effects in humans after oral exposure to organic arsenicals. The developmental toxicity of organic arsenicals has been investigated in rats and rabbits for MMA and in rats, mice, and rabbits for DMA. Decreased fetal weights and an increased incidence of fetuses with incomplete ossification of thoracic vertebrae were observed in the offspring of rats administered via gavage 500 mg MMA/kg/day on gestational days 6–15; no

developmental effects were observed at 100 mg MMA/kg/day (Irvine et al. 2006). Decreases in maternal body weight gain were observed at 100 and 500 mg MMA/kg/day. A decrease in pup survival was observed in F_1 and F_2 offspring of rats exposed to 76 mg MMA/kg/day (Schroeder 1994); although pup survival was not statistically different from controls, the investigators considered the effect to be biologically significant because survival in the MMA pups was outside the lower range of survival in historical controls. Increases in the number of fetuses with supernumerary thoracic ribs and eight lumbar vertebrae were observed in the offspring of rabbits administered to 12 mg MMA/kg/day on gestational days 7–19 (Irvine et al. 2006); the investigators noted that these effects were probably secondary to maternal stress.

No developmental effects were observed in the offspring of rats administered via gavage 15 mg DMA/kg/day on gestational days 7–16 (Rogers et al. 1981). At 30 mg DMA/kg/day, there was an increase in the percentage of fetuses with irregular palatine rugae; no maternal effects were observed at this dose level (Rogers et al. 1981). The investigators noted that the functional significance of aberrant rugae in rats is not known. Doses of ≥36 mg DMA/kg/day resulted in decreases in fetal weights and delays in ossification (Chernoff et al. 1990; Irvine et al. 2006; Rogers et al. 1981); decreases in maternal body weight gain were often observed at the same dose levels. Irvine et al. (2006) also reported an increase in the occurrence of diaphragmatic hernia in the offspring of rats exposed to 36 mg DMA/kg/day as DMA on gestational days 6–15. Mice appear to be less sensitive than rats to the developmental toxicity of DMA. No developmental effects were observed in the offspring of mice administered 200 mg DMA/kg/day on gestational days 7–16 (Rogers et al. 1981); at higher doses, decreases in fetal body weight, delays in ossification, and cleft palate were observed (Kavlock et al. 1985; Rogers et al. 1981). In rabbits, a NOAEL of 12 mg DMA/kg/day was identified (Irvine et al. 2006); at 48 mg DMA/kg/day, there were increased maternal deaths and abortions.

3.2.2.7 Cancer

Inorganic Arsenicals. There is convincing evidence from a large number of epidemiological studies and case reports that ingestion of inorganic arsenic increases the risk of developing skin cancer (Alain et al. 1993; Beane Freeman et al. 2004; Bickley and Papa 1989; Cebrián et al. 1983; Chen et al. 2003; Guo et al. 2001a; Haupert et al. 1996; Hsueh et al. 1995; Lewis et al. 1999; Lüchtrath 1983; Mitra et al. 2004; Morris et al. 1974; Piontek et al. 1989; Sommers and McManus 1953; Tay and Seah 1975; Tsai et al. 1998a, 1999; Tseng 1977; Tseng et al. 1968; Zaldívar 1974; Zaldívar et al. 1981). Lesions commonly observed are multiple squamous cell carcinomas, some of which appear to develop from the

hyperkeratotic warts or corns described in Section 3.2.2.2. In addition, multiple basal cell carcinomas may occur, typically arising from cells not associated with hyperkeratinization. In most cases, skin cancer develops only after prolonged exposure, but one study has reported skin cancer in people exposed for <1 year (Reymann et al. 1978). Although both types of skin cancer can be removed surgically, they may develop into painful lesions that may be fatal if left untreated (Shannon and Strayer 1989).

A number of studies that identify CELs in exposed humans are summarized in Table 3-3 and shown in Figure 3-3. The EPA reviewed the studies that provided dose-response data on the risk of skin cancer (EPA 1988d) and concluded that the most useful study for the purposes of quantitative risk assessment was the ecologic epidemiology study by Tseng et al. (1968). In this study, the incidence of skin cancer was measured as a function of exposure level in over 40,000 people residing in 37 villages in Taiwan, and compared to a control group of over 7,500 people. Beyond the very large sample size, other strengths of this study include excellent case ascertainment (physical examination), inclusion of both males and females, and lifetime exposure duration. Weaknesses and uncertainties include poor nutritional status of the exposed populations, their genetic susceptibility, their exposure to inorganic arsenic from nonwater sources, and the applicability of extrapolating data from Taiwanese to the U.S. population because of different background rates of cancer, possibly genetically determined, and differences in diet other than arsenic (e.g., low protein and fat and high carbohydrate) (EPA 1988d). Because of a lack of information on the amount of individual exposure, subjects were classified into three exposure groups (i.e., high, medium, and low). Based upon pooled data for skin cancer incidence and average well concentrations for each village in the Tseng et al. (1968) study, the EPA calculated a unit risk (the upper-bound excess cancer risk from lifetime exposure to water containing 1 μg As/L) of $5x10^{-5}$ (IRIS 2007). The average daily doses (expressed as mg As/kg/day) that correspond to excess cancer risks of $1x10^{-4}$–$1x10^{-7}$ are shown in Figure 3-3.

The use of a cancer risk estimate derived from the Tseng et al. (1968) study for a U.S. population has been the source of intense debate. Some have argued and have provided data in support of the view that there is persuasive evidence that inorganic arsenic is a cause of human cancer at several sites (i.e., Smith et al. 1992, 1995, 2002). On the other hand, a number of concerns have been raised regarding the strength, or lack of strength, of the database, including: the adequacy of the model used by EPA and the accuracy and reliability of the exposure data (Brown et al. 1997a, 1997b); a number of host and environmental factors among the Taiwanese not applicable elsewhere (Carlson-Lynch et al. 1994); a possible threshold for arsenic carcinogenicity and nonlinearities in the dose-response curve (Abernathy et al. 1996; Slayton et al. 1996); differences in health and nutrition between Taiwan and the United States

that might increase cancer risk in Taiwan (Beck et al. 1995); the possibility that lower doses of arsenic may be beneficial role in some physiological processes (EPA 1988d; FNB/IOM 2001; NRC 1999, 2001); and the possibility of significant exposure to arsenic from sources other than the well water (Chappell et al. 1997). Many of these factors were recognized by EPA (1988d). A report by NRC (2001) suggested that the risks calculated based on increases in incidence of lung and bladder cancers may be greater than those calculated by the EPA based on incidences of skin cancer.

Several early epidemiological studies performed in the United States did not report an increased frequency of skin cancer in small populations consuming water containing arsenic at levels of around 0.1–0.2 ppm (EPA 1981b; Goldsmith et al. 1972; Harrington et al. 1978; Morton et al. 1976). These early data suggested that arsenic-associated skin cancer is not a common problem in this country, but these studies lacked sufficient statistical power to detect small increases in skin cancer incidence that might have occurred at these low doses (EPA 1983g). Later studies in exposed U.S. populations from Utah (Lewis et al. 1999) and Iowa (Beane Freeman et al. 2004) have suggested that arsenic-exposed individuals within the United States may have increased incidence or risk of mortality from some skin cancers, melanoma in particular; however, exposure data from these studies are generally insufficient for dose-response analysis. Another study found a suggestion of an arsenic-induced effect on the development of skin cancer, but the association did not achieve statistical significance (Karagas et al. 2001). Therefore, the risk of arsenic-induced skin cancers in U.S. populations, while it may appear to be less than in some other evaluated populations, may be the reflection that, in most studies, exposures were lower.

In addition to the risk of skin cancer, there is mounting evidence that ingestion of arsenic may increase the risks of internal cancers as well. Many case studies have noted the occurrence of internal tumors of the liver and other tissues in patients with arsenic-induced skin cancer (Falk et al. 1981b; Kasper et al. 1984; Koh et al. 1989; Lander et al. 1975; Regelson et al. 1968; Sommers and McManus 1953; Tay and Seah 1975; Zaldívar et al. 1981). These studies are supported by large-scale epidemiological studies, where associations and/or dose response trends have been detected for tumors of the bladder, kidney, liver, lung, and prostate (Chen and Wang 1990; Chen et al. 1985, 1986, 1988a, 1988b, 1992; Chiou et al. 1995; Cuzick et al. 1992; Ferreccio et al. 1998; Guo et al. 1997; Hopenhayn-Rich et al. 1998; Kurttio et al. 1999; Lewis et al. 1999; Moore et al. 2002; Rivara et al. 1997; Smith et al. 1998; Tsuda et al. 1995a; Wu et al. 1989). The EPA has not yet calculated a unit risk value or slope factor for arsenic-induced internal tumors.

There is increasingly convincing evidence that long-term exposure to arsenic can result in the development of bladder cancer (Bates et al. 2004; Chen et al. 1992, 2003; Chiou et al. 1995, 2001; Cuzick et al. 1992; Guo et al. 2001b; Karagas et al. 2004; Lamm et al. 2004; Michaud et al. 2004; Steinmaus et al. 2003), with transitional cell cancers being the most prevalent. Chiou et al. (1995) reported a dose-response relationship between long-term arsenic exposure from drinking artesian well water and the incidence of lung cancer, bladder cancer, and cancers of all sites combined (after adjustment for age, sex, and cigarette smoking) in four townships in Taiwan exposed to inorganic arsenic in drinking water (0–1.14 mg/L). In a later followup study of the same cohort, the increase in bladder cancer was found to be statistically significant only in subjects exposed for 40 years or longer (Chiou et al. 2001). Cuzick et al. (1992) evaluated a cohort treated with Fowler's solution (potassium arsenite) in Lancashire, England, during the period 1945–1969 and followed through 1991; the cohort of 478 patients showed a significant excess of bladder cancer, but no excess for other causes of death. Of a subcohort of 142 patients examined for signs of arsenicism around 1970 (Cuzick et al. 1992), all 11 subsequent cancer deaths occurred in those with signs of arsenicism (p=0.0009). Hopenhayn-Rich et al. (1996a) investigated bladder cancer mortality for the years 1986–1991 in the 26 counties of Cordoba, Argentina, and reported that bladder cancer SMRs were consistently higher in counties with documented arsenic exposure; a later case-control study by the same authors (Bates et al. 2004) did not report statistically significant increases in bladder cancers resulting from arsenic exposure, except in individuals exposed for 50 years or longer. Guo et al. (2001a) reported significantly increased rate differences for bladder cancer in men and women in Taiwan exposed to 0.64 mg arsenic/L in the drinking water, but not at lower exposure levels. The arsenic-induced bladder tumors do not appear to be histologically different than similar bladder tumor types of nonarsenic origin (Chow et al. 1997), although they tended to be more pronounced. In contrast, Michaud et al. (2004) reported no correlation between arsenic levels in toenails and the incidence of bladder cancers in Finnish workers. Among evaluated U.S. cohorts, there has generally been no association between arsenic exposure (~60–100 μg As/L) and the incidence of mortality from bladder cancers (Lamm et al. 2004; Steinmaus et al. 2003), although it is possible that smoking may render individuals more susceptible to arsenic-induced bladder tumors (Karagas et al. 2004; Steinmaus et al. 2003).

Studies have also suggested that chronic oral exposure to arsenic may result in the development of respiratory tumors and increased incidence of lung cancer (Ferreccio et al. 2000; Guo 2004; Nakadaira et al. 2002; Smith et al. 1998; Viren and Silvers 1999). A study of arsenic-exposed individuals in northern Chile reported significantly increased odds ratios for lung cancer among subjects with ≥30 μg As/L of drinking water (Ferreccio et al. 2000), although when adjusted for socioeconomic status, smoking, and

other factors, the increase was only significant at 60 μg As/L or greater. Guo (2004) reported significantly increased rates differences (RD) for lung cancer for Taiwanese men and women exposed to 0.64 mg As/L or greater, with those subjects >50 years of age being particularly at risk. Nakadaira et al. (2002) suggested that even comparatively short exposure durations (≤5 years) may be sufficient for the development of arsenic-induced lung cancer.

Studies in U.S. populations exposed to arsenic in drinking water (EPA 1981b; Lamm et al. 2004; Lewis et al. 1999; Morton et al. 1976; Steinmaus et al. 2003; Valentine et al. 1992) have not yielded the cancer incidences and health effects noted in Taiwan, Mexico, and Chile. Whether this difference is due to a smaller population of subjects compared to Taiwan, to overall lower doses in exposed U.S. populations, or to differences in nutritional or socioeconomic conditions has not been resolved. It should be noted that exposed populations in Mexico and Chile are also smaller than those in Taiwan.

Most studies of animals exposed to arsenate or arsenite by the oral route have not detected any clear evidence for an increased incidence of skin cancer or other cancers (Byron et al. 1967; Kroes et al. 1974; Schroeder et al. 1968). Arsenic has sometimes been called a “paradoxical” human carcinogen because of this lack of animal data (Jager and Ostrosky-Wegman 1997). The basis for the lack of tumorigenicity in animals is not known, but could be related to species-specific differences in arsenic distribution, and induction of cell proliferation (Byrd et al. 1996) (see Section 3.5). As discussed in Section 3.5 below, the carcinogenic effects of arsenic may partially result from its function as a cocarcinogen, which would not manifest in most animal carcinogenicity studies.

One mouse study using transgenic mice (which carry the v-Ha-ras oncogene) administered 48 mg As/kg/day as sodium arsenite in drinking water for 4 weeks followed by dermal application of 12-O-tetradecanoylphorbol-13-acetage (TPA) to shaved back skin twice a day for 2 weeks showed an increase in the incidence of skin papillomas when compared to transgenic mice receiving only TPA treatment, only arsenic, or to wild-type mice receiving both TPA and arsenic (Germolec et al. 1998); arsenic treatment alone did not result in increased papilloma incidence. Increases in mRNA transcripts for the growth factors transforming growth factor-α (TGF-α) and granulocyte/ macrophage-colony stimulating factor (GM-CSF) were detected in the epidermis of the arsenic-treated mice.

A few studies in mice have noted that arsenic ingestion may actually decrease the incidence of some tumor types. For example, arsenic exposure caused decreased incidence of urethane-induced pulmonary tumors (Blakley 1987), spontaneous mammary tumors (Schrauzer and Ishmael 1974; Schrauzer et al.

1976), and tumors resulting from injection of mouse sarcoma cells (Kerkvliet et al. 1980). However, arsenic also increased the growth rate of the tumors that did occur, resulting in a net decrease in survival time in tumor-bearing animals (Kerkvliet et al. 1980; Schrauzer and Ishmael 1974). These observations suggest that arsenic may affect different types of neoplastic cells differently, perhaps acting mainly as a tumor promoter (Schrauzer and Ishmael 1974; Shirachi et al. 1983), although some studies have suggested that arsenic's actions are not consistent with tumor promotion (Baroni et al. 1963; Boutwell 1963).

There is evidence suggesting that inorganic arsenic can induce cancer in the offspring from mice exposed to arsenic during gestation (transplacental carcinogen) (Waalkes et al. 2003, 2004a, 2004b, 2004c, 2006a, 2006b). These studies are summarized in Section 3.2.2.6, Developmental Effects.

Organic Arsenicals. No studies were located regarding cancer in humans after oral exposure to organic arsenicals. Two lifetime carcinogenicity studies with MMA did not find significant increases in tumors in rats exposed to 72.4 mg MMA/kg/day in the diet for 2 years (Arnold et al. 2003) or 8.4 mg MMA/kg/day in drinking water for 2 years (Shen et al. 2003). No significant increases in neoplastic lesions were observed in mice exposed to 67.1 mg MMA/kg/day in the diet for 2 years (Arnold et al. 2003).

In contrast, significant increases in the incidence of urinary bladder tumors have been observed in rats exposed for 2 years to 7.8 mg DMA/kg/day in the diet (Arnold et al. 2006) or 3.4 mg DMA/kg/day in drinking water (Wei et al. 1999, 2002). The incidence of bladder tumors was similar to controls in the rats exposed to 0.77 mg DMA/kg/day (Arnold et al. 2006) or 0.75 mg DMA/kg/day (Wei et al. 1999, 2002). Neither study reported significant increases in the incidence of neoplastic lesions in other tissues. Arnold et al. (2006) did not find increases in the incidence of neoplastic lesions in mice exposed to doses as high as 94 mg DMA/kg/day in the diet for 2 years. Hayashi et al. (1998) reported that exposure of A/J mice (a strain susceptible to lung tumorigenesis) to 10.4 mg DMA/kg/day (but not 1.3 or 5.2 mg DMA/kg/day) in drinking water for 50 weeks resulted in an increased incidence of papillary adenomas and/or adenocarcinomas and an increased number of lung tumors per mouse.

The incidence of basophilic foci (believed to be a precancerous lesion) in the liver of rats initiated with diethylnitrosamine was increased by subsequent 6-month drinking water exposure to 11 mg DMA/kg/day, suggesting that this compound could act as a cancer promoter (Johansen et al. 1984). Additional evidence for the possible role of DMA as a promoter comes from Yamamoto et al. (1995), who reported that 15 or 60 mg DMA/kg/day in the drinking water for 24 weeks significantly enhanced the tumor induction in the

urinary bladder, kidney, liver, and thyroid gland in male F344 rats treated with a series of initiators. Wanibuchi et al. (1996) reported that treatment of F344 rats for 32 weeks with up to 14.3 mg DMA/kg/day DMA in the drinking water did not result in increased incidences of urinary bladder papillomas or carcinomas, but that incidence of these tumors was elevated if the animals were first pretreated with an initiating compound (BBN). A later study by Li et al. (1998) reported that NBR rats (which do not synthesize $\alpha_{2\mu}$-globulin) exposed to an initiator for 4 weeks followed by DMA for 32 weeks, similar to the Wanibuchi et al. (1996) study, showed a statistically significant increase in simple hyperplasia and papillary or nodular hyperplasia of the bladder. A study by Salim et al. (2003) suggested that DMA primarily exerts its carcinogenic effects on spontaneous tumor development.

No increases in tumor incidence were observed in rats, mice, or dogs exposed to 10, 13, or 5 mg/kg/day roxarsone, respectively, in the diet for 2 years (Prier et al. 1963). Similarly, no evidence of carcinogenicity was observed in female rats or male or female mice exposed to 4 or 43 mg/kg/day as roxarsone in the diet for 2 years (NTP 1989b). However, a slight increase in pancreatic tumors was noted in male rats exposed to 4 mg/kg/day (NTP 1989b); this was considered to constitute equivocal evidence of carcinogenicity.

3.2.3 Dermal Exposure

Adverse effects from dermal exposure to inorganic or organic arsenicals have not been extensively investigated. Table 3-7 summarizes studies in animals and humans that provide quantitative data on dermal exposure-effect relationships for inorganic arsenicals. No quantitative data on dermal exposure to organic arsenicals were located. Available quantitative and qualitative data are discussed in greater detail below.

3.2.3.1 Death

Inorganic Arsenicals. No studies were located regarding death in humans after dermal exposure to inorganic arsenicals. In rats, no deaths resulted from dermal exposure to arsenate or arsenite at doses up to 1,000 mg As/kg (Gaines 1960). These data indicate that dermal exposure to inorganic arsenic compounds is very unlikely to result in death.

Organic Arsenicals. No studies were located regarding death in humans after dermal exposure to organic arsenicals. No deaths were observed in rabbits receiving daily dermal applications of 540 mg As/kg as

Table 3-7 Levels of Significant Exposure to Inorganic Arsenic - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference Chemical Form	Comments
ACUTE EXPOSURE							
Immuno/ Lymphoret							
Gn Pig (Hartley)	once		580 mg/L			Wahlberg and Boman 1986 As(+3)	
Gn Pig (Hartley)	once		4000 mg/L			Wahlberg and Boman 1986 As(+5)	
INTERMEDIATE EXPOSURE							
Systemic							
Mouse (Rockland)	30 wk 11 x/wk	Dermal		6 F mg/kg/day	(gross hyperplasia, ulceration)	Boutwell 1963 As(+3)	

F = female; Gn pig = guinea pig; Immuno/Lymphoret = immunological/lymphoreticular; wk = week(s); x = time(s)

MMA 5 days/week for 21 days (Margitich and Ackerman 1991b) or 1,000 mg DMA/kg/day 5 days/week for 21 days (Margitich and Ackerman 1991a).

3.2.3.2 Systemic Effects

No studies were located that have associated respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, endocrine, ocular, or body weight effects in humans or animals with dermal exposure to inorganic arsenicals.

Respiratory Effects. No studies were located regarding respiratory effects in humans after dermal exposure to organic arsenicals. No histological effects were observed in the respiratory tracts of rabbits following dermal application of 1,000 mg/kg/day MMA or DMA 5 days/week for 21 days (Margitich and Ackerman 1991a, 1991b).

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after dermal exposure to organic arsenicals. No histological effects were observed in the hearts of rabbits following dermal application of 1,000 mg/kg/day MMA or DMA 5 days/week for 21 days (Margitich and Ackerman 1991a, 1991b).

Hematological Effects. No studies were located regarding hematological effects in humans after dermal exposure to organic arsenicals. No treatment-related hematological alterations were observed in rabbits receiving dermal applications of 1,000 mg MMA/kg/day (Margitich and Ackerman 1991a) or 1,000 mg DMA/kg/day 5 days/week for 21 days (Margitich and Ackerman 1991b).

Hepatic Effects. No studies were located regarding hepatic effects in humans after dermal exposure to organic arsenicals. No significant alterations in blood clinical chemistry, liver weights, or histopathology were observed in rabbits dermally exposed to 1,000 mg/kg/day MMA or DMA 5 days/week for 21 days (Margitich and Ackerman 1991a, 1991b).

Renal Effects. No studies were located regarding renal effects in humans after dermal exposure to organic arsenicals. No significant alterations in urinalysis, kidney weights, or histopathology were observed in rabbits following dermal exposure to 1,000 mg MMA/kg/day (Margitich and Ackerman 1991a) or 1,000 mg DMA/kg/day 5 days/week for 21 days (Margitich and Ackerman 1991b).

Endocrine Effects. No studies were located regarding endocrine effects in humans after dermal exposure to organic arsenicals. No alterations in adrenal gland weight or histopathology of the adrenal glands, pancreas, pituitary gland, thyroid gland, and parathyroid gland were observed in rabbits following dermal application of 1,000 mg/kg/day MMA or DMA 5 days/week for 21 days (Margitich and Ackerman 1991a, 1991b).

Dermal Effects.

Inorganic Arsenicals. Several studies of humans exposed to arsenic dusts in the workplace have reported that inorganic arsenic (usually arsenic trioxide) can cause contact dermatitis (Holmqvist 1951; Pinto and McGill 1953). Typical responses included erythema and swelling, with papules and vesicles in more severe cases (Holmqvist 1951). The dermal contact rates that cause these effects in humans have not been quantified, but a similar direct irritation of the skin has been noted in mice exposed to 4 mg As/kg/day as potassium arsenite for 30 weeks (Boutwell 1963). In contrast, no significant dermal irritation was noted in guinea pigs exposed to aqueous solutions containing 4,000 mg As/L as arsenate or 580 mg As/L as arsenite (Wahlberg and Boman 1986). These studies indicate that direct contact may be of concern at high exposure levels, but do not suggest that lower levels are likely to cause significant irritation.

Studies on possible dermal sensitization by inorganic arsenicals are discussed in Section 3.2.3.3 below.

Organic Arsenicals. Contact dermatitis was reported in workers involved in the application of an organic arsenical herbicide, which is a mixture of DMA and its sodium salt (Peoples et al. 1979).

Application of an unspecified amount of MMA to the skin of rabbits was reported to result in mild dermal irritation in a Draize test (Jaghabir et al. 1988). No dermal irritation was reported in rabbits repeatedly exposed to 1,000 mg MMA/kg/day (Margitich and Ackerman 1991a) or 1,000 mg DMA/kg/day 5 days/week for 21 days (Margitich and Ackerman 1991b).

Ocular Effects. No studies were located regarding ocular effects in humans or animals after dermal exposure to organic arsenicals.

Body Weight Effects. No studies were located regarding body weight effects in humans after dermal exposure to organic arsenicals. No significant alterations in body weight gain were observed in rabbits

following a 5 day/week exposure to 1,000 mg/kg/day MMA or DMA for 21 days (Margitich and Ackerman 1991a, 1991b).

3.2.3.3 Immunological and Lymphoreticular Effects

Inorganic Arsenicals. Examination of workers exposed to arsenic trioxide dusts in a copper smelter led Holmqvist (1951) to suspect that repeated dermal contact could lead to dermal sensitization. In support of this, Holmqvist (1951) found a positive patch test in 80% of the exposed workers compared to 30% in a control population. These data do suggest that workers may be sensitized to arsenic, but the high response rate in controls seems unusual. A much lower response rate (0.5%) was noted in another patch test study of dermal sensitization (Wahlberg and Boman 1986), and the few positive responses seemed to be due to a cross-reactivity with nickel. Mohamed (1998) evaluated 11 male workers at a tin smelting factory where arsenic trioxide levels ranged from 5.2 to 14.4 mg/m^3. The workers experienced symptoms of generalized itch, dry and hyperpigmented skin, folliculitis, and superficial ulcerations. The authors concluded that arsenic-containing dust collected on the sweat on the workers' skin, causing contact dermatitis. Studies in guinea pigs did not yield evidence of a sensitization reaction to inorganic arsenic (Wahlberg and Boman 1986).

Organic Arsenicals. Support for sensitization to DMA is provided in a case report of a 26-year-old woman who was occupationally exposed to DMA and experienced eczema on her face (Bourrain et al. 1998). Patch testing confirmed an allergic reaction to DMA, and avoidance of DMA resulted in disappearance of the symptoms. No studies were located regarding immunological or lymphoreticular effects in animals after dermal exposure to organic arsenicals.

No studies were located that have associated any of the following effects in humans or animals with dermal exposure to inorganic or organic arsenicals:

3.2.3.4 Neurological Effects

3.2.3.5 Reproductive Effects

3.2.3.6 Developmental Effects

3.2.3.7 Cancer

Inorganic Arsenicals. No studies were found that have associated cancer in humans with dermal exposure to arsenic. Application of arsenic acid to the skin of mice pretreated with dimethylbenz-

anthracene did not result in any skin tumors (Kurokawa et al. 1989), suggesting that arsenic does not act as a promoter in this test system.

Organic Arsenicals. No studies were located regarding cancer in humans or animals after dermal exposure to organic arsenicals.

3.3 GENOTOXICITY

Inorganic Arsenicals. There have been a large number of studies of the genotoxic effects of arsenic. Tables 3-8 and 3-9 summarize a number of reports on the *in vitro* and *in vivo* genotoxicity of inorganic arsenicals, respectively. In general, *in vitro* studies in prokaryotic organisms have been negative for gene mutations (Lantzsch and Gebel 1997; Löfroth and Ames 1978; Nishioka 1975; Rossman et al. 1980; Ulitzur and Barak 1988). Studies in human fibroblasts, lymphocytes, and leukocytes, mouse lymphoma cells, Chinese hamster ovary cells, and Syrian hamster embryo cells demonstrate that *in vitro* arsenic exposure can induce chromosomal aberrations and sister chromatid exchange (see Table 3-8 for citations). *I vitro* studies in human, mouse, and hamster cells have also been positive for DNA damage and repair and enhancement or inhibition of DNA synthesis.

Studies of humans have detected a higher-than-average incidence of chromosomal aberrations in peripheral lymphocytes, both after inhalation exposure (Beckman et al. 1977; Nordenson et al. 1978) and oral exposure (Burgdorf et al. 1977; Nordenson et al. 1979). These studies must be interpreted with caution, since in most cases, there were only a small number of subjects and a number of other chemical exposures were possible (EPA 1984a). Human and animal data are available indicating that inhaled inorganic arsenic is clastogenic. Workers exposed to unspecified concentrations of arsenic trioxide at the Ronnskar copper smelter in Sweden were found to have a significant increase in the frequency of chromosomal aberrations in peripheral lymphocytes (Beckman et al. 1977; Nordenson et al. 1978). This result is supported by an animal study that found increased chromosomal aberrations in the livers of fetuses from pregnant mice exposed to 22, but not 2.2 or 0.20, mg As/m^3 as arsenic trioxide on days 9–12 of gestation (Nagymajtényi et al. 1985). Workers in the arsenic-based glass making industry in southern India had a significantly increased frequency of micronuclei in buccal cells and increased DNA damage in leukocytes compared to a control group (Vuyyuri et al. 2006). Exposure levels were not available, but the concentration of arsenic in the blood from workers was approximately 5 times higher than in the reference group.

Table 3-8. Genotoxicity of Inorganic Arsenic *In Vitro*

Valence	Species (test system)	End point	Results: With activation	Results: Without activation	Reference
Prokaryotic organisms:					
As^{+3}	*Escherichia coli*	Reverse mutation	No data	+	Nishioka 1975
As^{+3}	*E. coli* PQ37	Gene mutation	No data	–	Lantzsch and Gebel 1997
As^{+3}	*E. coli* (six strains)	Reverse mutation	No data	–	Rossman et al. 1980
As^{+3}	*Salmonella typhimurium*	Gene mutation	No data	–	Löfroth and Ames 1978
As^{+3}	*Photobacterium fischeri*	Gene mutation	No data	–	Ulitzur and Barak 1988
As^{+5}	*S. typhimurium*	Gene mutation	No data	–	Löfroth and Ames 1978
As^{+5}	*P. fischeri*	Gene mutation	No data	+	Ulitzur and Barak 1988
Eukaryotic organisms:					
Fungi:					
As^{+3}; As^{+5}	*Saccharomyces cerevisiae*	Gene mutation	No data	–	Singh 1983
	Mammalian cells:				
As^{+3}	Human fibroblasts	DNA repair inhibition	No data	+	Okui and Fujiwara 1986
As^{+3}	Human fibroblasts	DNA repair and mutant frequencies	+	+	Wiencke et al. 1997
As^{+3}	Human fibroblasts	DNA repair inhibition	+	+	Hartwig et al. 1997
As^{+3}	Human fibroblasts (MRC5CV1)	DNA migration	No data	+	Hartmann and Speit 1996
As^{+3}	Human fibroblasts (HFW cells)	Cytotoxicity	No data	+	Lee and Ho 1994
As^{+3}	Human skin fibroblasts (HFW)	Chromosome endoreduplication	No data	+	Huang et al. 1995
As^{+3}	Human skin fibroblasts	Chromosomal aberrations	No data	+	Yih et al. 1997
As^{+3}	Human fetal lung fibroblasts	DNA strand breaks	No data	+	Dong and Luo 1993
As^{+3}	Human fetal lung fibroblasts (2BS cells)	DNA damage and repair	No data	+	Dong and Luo 1994
As^{+3}; As^{+5}	Human umbilical cord fibroblasts	Chromosomal aberrations	No data	+	Oya-Ohta et al. 1996
As^{+3}	Diploid human fibroblasts	Morphological transformation	No data	+	Landolph 1994

Table 3-8. Genotoxicity of Inorganic Arsenic *In Vitro*

Valence	Species (test system)	End point	Results		Reference
			With activation	Without activation	
As^{+3}	Human leukocytes	Chromosomal aberration	No data	+	Nakamuro and Sayato 1981
As^{+3}	Human lymphocytes	DNA protein cross-links	–	–	Costa et al. 1997
As^{+3}; As^{+5}	Human lymphocytes	Enhancement or inhibition on DNA synthesis	No data	+	Meng 1993a
As^{+3}; As^{+5}	Human lymphocytes	Enhancement or inhibition on DNA synthesis	No data	+	Meng 1993b
As^{+3}; As^{+5}	Human lymphocytes	Enhancement or inhibition on DNA synthesis	No data	+	Meng 1994
As^{+3}	Human lymphocytes	Hyperdiploidy and chromosomal breakage	No data	(+)	Rupa et al. 1997
As^{+3}	Human lymphocytes	Hyperdiploid nuclei	No data	+	Ramirez et al. 1997
As^{+3}	Human lymphocytes	Chromosomal aberration	No data	+	Beckman and Nordenson 1986
As^{+3}	Human lymphocytes	Chromosomal aberrations and sister chromatid exchange	No data	+	Nordenson et al. 1981
As^{+3}	Human lymphocytes	Chromosomal aberration	No data	+	Sweins 1983
As^{+3}	Human lymphocytes	Chromosomal aberrations	No data	+	Yager and Wiencke 1993
As^{+3}	Human lymphocytes	Chromosomal aberrations	No data	+	Vega et al. 1995
As^{+3}	Human lymphocytes	Chromosomal aberrations	No data	+	Wan et al. 1982
As^{+3}	Human lymphocytes	Chromosomal aberrations and sister chromatic exchange	No data	+	Wiencke and Yager 1992
As^{+3}	Human lymphocytes	Chromosome aberrations and sister chromatid exchanges	No data	+	Larramendy et al. 1981
As^{+3}	Human lymphocytes	Sister chromatid exchange	No data	+	Gebel et al. 1997
As^{+5}	Human lymphocytes	Sister chromatid exchange	No data	–	Gebel et al. 1997
As^{+3}	Human lymphocytes	Sister chromatid exchange	No data	+	Hartmann and Speit 1994
As^{+3}	Human lymphocytes	Sister chromatid exchange	No data	+	Jha et al. 1992
As^{+3}	Human lymphocytes	Sister chromatid exchange	No data	+	Rasmussen and Menzel 1997

Table 3-8. Genotoxicity of Inorganic Arsenic *In Vitro*

Valence	Species (test system)	End point	Results: With activation	Results: Without activation	Reference
As^{+3}; As^{+5}	Human T-cell lymphoma-derived cell line (Molt-3)	PARP activity inhibition	No data	+	Yager and Wiencke 1997
As^{+3}	Human cervix carcinoma HeLa and cisplatin-resistant HeLa/CPR variant cells	DNA repair modification	+	+	Chao 1996
As^{+3}	Human cervix carcinoma cells (HeLa)	DNA damage recognition	No data	-	Hartwig et al. 1998
As^{+3}	Human osteosarcoma cells (HOS)	DNA repair	No data	+	Hu et al. 1998
As^{+3}	Human osteosarcoma cells (HOS)	Cell transformation	No data	+	Mure et al. 2003
As^{+3}	Human-hamster hybrid A_1 cells	DNA adducts	No data	+	Kessel et al. 2002
As^{+3}	Mouse lymphoma cells	Enhanced viral forward mutation	No data	(+)	Oberly et al. 1982
As^{+3}; As^{+5}	Mouse lymphoma cells [L5178Y/TK$^{+/-}$ (-3.7.2C)]	Chromosomal mutations	No data	+	Moore et al. 1997a
As^{+3}	Mouse lymphoma cells [L5178Y tk$^{+/-}$ (3.7.sc)]	Mutagenicity	No data	+	Oberly et al. 1996
As^{+3}; As^{+5}	Mouse lymphoma cells	Chromosomal aberrations	No data	+	Moore et al. 1994a
As^{+3}	Mouse lymphoma cells	Chromosomal aberrations	No data	+	Sofuni et al. 1996
As^{+3}	Mouse 3T6 cells	Gene amplification	No data	+	Lee et al. 1988
As^{+3}	Mouse embryo fibroblasts (C3H/10T/2 Cl8)	Morphological transformation	No data	+	Landolph 1994
As^{+3}	Chinese hamster V79 cells	Gene mutation	No data	–	Li and Rossman 1991
As^{+3}	Chinese hamster V79 cells	Gene mutation	No data	–	Rossman et al. 1980
As^{+3}	Chinese hamster V79 cells	DNA damage, DNA-protein cross-linking, micronucleus induction	No data	+	Gebel et al. 1998a
As^{+3}	Chinese hamster V79 cells	DNA repair and mutant frequencies	No data	+	Li and Rossman 1991
As^{+3}	Chinese hamster V79 cells	Intrachromosomal homologous recombination	No data	+	Helleday et al. 2000
As^{+3}	Chinese hamster ovary cells (CHO-AL)	Gene mutation	No data	+	Hei et al. 1998
As^{+3}	Chinese hamster ovary cells (CHO-AS52)	Mutagenicity	No data	+	Meng and Hsie 1996

Table 3-8. Genotoxicity of Inorganic Arsenic *In Vitro*

Valence	Species (test system)	End point	Results: With activation	Results: Without activation	Reference
As^{+3}	Chinese hamster ovary cells	Gene mutation	No data	+	Yang et al. 1992
As^{+3}	Chinese hamster ovary cells	DNA repair inhibition	No data	+	Lee-Chen et al. 1993
As^{+3}	Chinese hamster ovary cells	DNA repair inhibition	No data	–	Lee-Chen et al. 1992
As^{+3}	Chinese hamster ovary cells (CHO-K1)	DNA strand breaks	+	+	Lee-Chen et al. 1994
As^{+3}	Chinese hamster ovary cells (CHO-K1)	DNA strand breaks	No data	+	Lynn et al. 1997
As^{+3}	Chinese hamster ovary cells	Aberrant metaphases	No data	+	Jan et al. 1986
As^{+3}	Chinese hamster ovary cells	Aberrant metaphases	No data	+	Lee et al. 1986
As^{+3}	Chinese hamster ovary cells	Chromosomal aberrations	+	+	Huang et al. 1992
As^{+3}	Chinese hamster ovary cells (CHO-K1)	Chromosomal aberrations	No data	+	Huang et al. 1993
As^{+3}; As^{+5}	Chinese hamster ovary cells (CHO-K1)	Chromosomal aberrations and sister chromatid exchange	No data	+	Kochhar et al. 1996
As^{+3}	Chinese hamster ovary cells	Chromosomal aberrations and sister chromatid exchange	+	+	Lin and Tseng 1992
As^{+3}	Chinese hamster ovary cells	Chromosomal aberrations and sister chromatid exchange	No data	+	Wan et al. 1982
As^{+3}	Chinese hamster ovary cells	Sister chromatid exchange and micronucleus induction	No data	+	Fan et al. 1996
As^{+3}	Chinese hamster ovary cells	Cell-killing and micronucleus induction	No data	+	Wang and Huang 1994
As^{+3}	Chinese hamster ovary cells	Micronuclei	No data	+	Liu and Huang 1997
As^{+3}	Chinese hamster ovary cells	Micronuclei formation	No data	+	Yee-Chien and Haimei 1996
As^{+3}	Chinese hamster ovary cells	Micronuclei induction	No data	+	Wang et al. 1997
As^{+3}	Chinese hamster ovary cells	Cytotoxicity	No data	–	Lee and Ho 1994
As^{+3}	Syrian hamster embryo cells	Gene mutation	No data	–	Lee et al. 1985

Table 3-8. Genotoxicity of Inorganic Arsenic *In Vitro*

Valence	Species (test system)	End point	Results: With activation	Results: Without activation	Reference
As^{+3}	Syrian hamster embryo cells	Chromosome aberrations and sister chromatid exchanges	No data	+	Larramendy et al. 1981
As^{+3}	Syrian hamster embryo cells	Chromosomal aberration	No data	+	Lee et al. 1985
As^{+3}	Syrian hamster embryo cells	Sister chromatid exchange	No data	+	Lee et al. 1985
As^{+3}	Syrian hamster embryo cells	Micronuclei induction	No data	–	Gibson et al. 1997
As^{+3}	Syrian hamster embryo cells	Micronuclei induction	No data	–	Gibson et al. 1997
As^{+3}	Syrian hamster embryo cells	Morphological transformation	No data	+	Kerckaert et al. 1996
As^{+3}	Syrian hamster embryo cells	Morphological transformation	No data	+	Lee et al. 1985
As^{+3}	Syrian hamster embryo cells	Morphological transformation	No data	+	Casto et al. 1979
As^{+5}	Human fibroblasts	DNA repair inhibition	No data	–	Okui and Fujiwara 1986
As^{+5}	Human leukocytes	Chromosomal aberrations	No data	(+)	Nakamuro and Sayato 1981
As^{+5}	Human lymphocytes	Chromosomal aberrations	No data	–	Nordenson et al. 1981
As^{+5}	Human lymphocytes	Chromosome aberrations and sister chromatid exchanges	No data	+	Larramendy et al. 1981
As^{+5}	Human lymphocytes	Sister chromatid exchange	No data	–	Rasmussen and Menzel 1997
As^{+5}	Human peripheral lymphocytes	Sister chromatid exchange	No data	+	Zanzoni and Jung 1980
As^{+5}	Human keratinocyte line SCC-9 cells	Keratinocyte programming and transcriptional activity	No data	+	Kachinskas et al. 1997
As^{+5}	Mouse lymphoma cells	Gene mutation	No data	–	Amacher and Paillet 1980
As^{+5}	Mouse lymphoma cells	Gene mutation	No data	–	Amacher and Paillet 1980
As^{+5}	Chinese hamster ovary cells	Chromosomal aberrations	No data	+	Wan et al. 1982
As^{+5}	Syrian hamster embryo cells	Gene mutation	No data	–	Lee et al. 1985
As^{+5}	Syrian hamster embryo cells	Chromosome aberrations and sister chromatid exchanges	No data	+	Larramendy et al. 1981

Table 3-8. Genotoxicity of Inorganic Arsenic *In Vitro*

Valence	Species (test system)	End point	Results		Reference
			With activation	Without activation	
As^{+5}	Syrian hamster embryo cells	Chromosomal aberrations	No data	+	Lee et al. 1985
As^{+5}	Syrian hamster embryo cells	Sister chromatid exchange	No data	+	Lee et al. 1985
As^{+5}	Syrian hamster embryo cells	Morphological transformation	No data	+	Lee et al. 1985
As^{+5}	Syrian hamster embryo cells	Morphological transformation	No data	+	DiPaolo and Casto 1979

(+) = weakly positive or marginal result; – = negative result; + = positive result; DNA = deoxyribonucleic acid

Table 3-9. Genotoxicity of Inorganic Arsenic *In Vivo*

Valence	Exposure route	Species (test system)	End point	Results	Reference
Nonmammalian					
As^{+3} As^{+5}	Injection	*Drosophila melanogaster*	Somatic mutations and mitotic recombination	+	Ramos-Morales and Rodriguez-Arnaiz 1995
As^{+3} As^{+5}	Larval feeding	*D. melanogaster*	Somatic mutations and mitotic recombination	+	Ramos-Morales and Rodriguez-Arnaiz 1995
As^{+5}	Larvae	*D. melanogaster*	Mitotic recombinations	+	de la Rosa et al. 1994
Mammalian					
As^{+3}	Inhalation	Human (lymphocytes)	Chromosomal aberrations	–	Beckman et al. 1977
As^{+3}	Inhalation	Human (lymphocytes)	Chromosomal aberrations	+	Nordenson et al. 1978
As^{+3}	Oral	Human (lymphocytes)	Chromosomal aberrations	–	Burgdorf et al. 1977
No data	Oral	Human (lymphocytes)	Chromosomal aberrations	–	Vig et al. 1984
No data	Oral	Human (skin)	DNA adducts	+	Matsui et al. 1999
As^{+3}	Oral	Human (lymphocytes)	Sister chromatid exchange	–	Burgdorf et al. 1977
As^{+3}	Oral	Human (lymphocytes)	Sister chromatid exchange	+	Hsu et al. 1997
No data	Oral	Human (lymphocytes)	Sister chromatid exchange	+	Lerda 1994
No data	Oral	Human (lymphocytes)	Sister chromatid exchange	+	Liou et al. 1999
No data	Oral	Human (lymphocytes)	Sister chromatid exchange	+	Mahata et al. 2003
As^{+3}	Oral	Human (lymphocytes)	Sister chromatid exchange	–	Nordenson et al. 1978
No data	Oral	Human (lymphocytes)	Sister chromatid exchange	–	Vig et al. 1984
No data	Oral	Human skin carcinoma	Mutation and overexpression of p53	+	Hsu et al. 1999
As^{+3}	Oral	Exfoliated human epithelial cells	Micronuclei	+	Moore et al. 1996
As^{+3}	Oral	Exfoliated human epithelial cells	Micronuclei	+	Tian et al. 2001
No data	Oral	Human (bladder cells)	Micronuclei	+	Moore et al. 1995
No data	Oral	Human (lymphocytes)	Micronuclei	+	Martínez et al. 2004
No data	Oral	Human (lymphocytes)	Micronuclei	+	Basu et al. 2004
No data	Oral	Human (oral mucosa cells)	Micronuclei	+	Basu et al. 2004

Table 3-9. Genotoxicity of Inorganic Arsenic *In Vivo*

Valence	Exposure route	Species (test system)	End point	Results	Reference
No data	Oral	Human (urothelial cells)	Micronuclei	+	Basu et al. 2004
As^{+5}	Oral	Rat (bone marrow cells)	Chromosomal aberrations	+	Datta et al. 1986
As^{+3}	Inhalation	Mouse (fetal liver)	Chromosomal aberrations	(+)	Nagymajtényi et al. 1985
As^{+3}	Oral	Mouse (bone marrow cells)	Chromosomal aberrations	+	Das et al. 1993
As^{+3}	Oral	Mouse (bone marrow cells)	Chromosomal aberrations	+	Poddar et al. 2000
As^{+3}	Oral	Mouse (bone marrow cells)	Chromosomal breaks, exchanges	–	Poma et al. 1987
As^{+3}	Oral	Mouse (spermatogonia)	Chromosomal aberrations	–	Poma et al. 1987
As^{+3}	Oral	Mouse (leukocytes)	Chromosomal breaks	+	McDorman et al. 2002
As^{+3}	Intraperitoneal	Mouse (bone marrow cells)	Chromosomal breaks, exchanges	–	Poma et al. 1981
As^{+3}	Intraperitoneal	Mouse (bone marrow cells)	Micronuclei	+	DeKnudt et al. 1986
As^{+3}	Intraperitoneal	Mouse (spermatogonia)	Spermatongonia	–	Poma et al. 1981
As^{+3}	Intraperitoneal	Mouse (spermatogonia)	Sperm morphology	–	DeKnudt et al. 1986
As^{+3}	Intraperitoneal	Mouse (spermatogenesis)	Dominant lethal mutations	–	DeKnudt et al. 1986

(+) = weakly positive or marginal result; – = negative result; + = positive result; DNA = deoxyribonucleic acid

Investigations of genotoxic effects of ingested arsenic have yielded mixed results possibly due to the different types of cells examined and the different exposure levels experienced by the populations studied. A study of p53 mutations in arsenic-related skin cancers from patients in Taiwan exposed to arsenic from drinking water found a high rate of p53 mutations and different types of p53 mutations compared with those seen in UV-induced skin cancers (Hsu et al. 1999); similar results have been found in mice (Salim et al. 2003). In humans exposed to Fowler's solution (potassium arsenite, usually taken at a dose of about 0.3 mg As/kg/day [Holland 1904]), increased sister chromatid exchanges, but no increase in chromosomal aberrations, was reported in one study (Burgdorf et al. 1977), while just the converse (increased aberrations but no increase in sister chromatid exchange) was reported in another (Nordenson et al. 1979). Moore et al. (1997a) reported an exposure-dependent increase in the occurrence of micronucleated cells in epithelial cells from the bladder in a male population in northern Chile chronically exposed to high and low arsenic levels in their drinking water (average concentrations, 600 and 15 μg As/L, respectively), and noted that chromosome breakage was the major cause of micronucleus (MN) formation. Similar results were reported by Martínez et al. (2004) who evaluated micronuclei formation in peripheral lymphocytes from people in northern Chile exposed to up to 0.75 mg As/L in their drinking water. In contrast, Martínez et al. (2005) did not find a significant increase in micronuclei in buccal cells from subjects from the same area relative to a low exposure group. Vig et al. (1984) found no significant differences in the frequency of chromosomal aberrations or sister chromatid exchanges between two populations in Nevada with differing levels of arsenic in their drinking water (mean concentrations of 5 and 109 μg/L). In animal studies, an increased incidence of chromosomal abnormalities was detected in rats given oral doses of sodium arsenate (4 mg As/kg/day) for 2–3 weeks (Datta et al. 1986), but no consistent increase in chromosomal aberrations was detected in bone marrow cells or spermatogonia from mice given sodium arsenite (about 50 mg As/kg/day) for up to 8 weeks (Poma et al. 1987). These studies suggest that ingested arsenic may cause chromosomal effects, but these data are too limited to draw a firm conclusion.

Organic Arsenicals. The genotoxicity of the organic arsenicals has been investigated in a number of studies (see Table 3-10). Several tests indicate that DMA and roxarsone may be able to cause chromosome aberrations, mutations, and deoxyribonucleic acid (DNA) strand breaks; *in vitro* studies with MMA did not find significant increases in the occurrence of chromosome aberrations, forward or reverse mutations, unscheduled DNA synthesis (Chun and Killeen 1989a, 1989b, 1989c, 1989d). An increased number of DNA strand breaks were detected in lung and other tissues of mice and rats given oral doses of ~1,500 mg/kg DMA (Okada and Yamanaka 1994; Yamanaka et al. 1989a); this effect appeared to be related to the formation of some active oxygen species. These breaks were largely repaired within 24 hours, so the relevance with respect to health risk is uncertain.

Table 3-10. Genotoxicity of Organic Arsenic

Chemical form	Species (test system)	End point	Results: With activation	Results: Without activation	Reference
Prokaryotic organisms (*in vitro*):					
MMA	*Salmonella typhimurium*	Gene mutation	–	–	Chun and Killeen 1989c
DMA	*Escherichia coli*	Gene mutation	No data	+	Yamanaka et al. 1989b
Roxarsone	*S. typhimurium*	Gene mutation	–	–	NTP 1989b
Eukaryotic organisms (*in vitro*):					
MMA	Chinese hamster ovary cells	Chromosome aberrations	–	–	Chun and Killeen 1989a
MMA	Mouse lymphoma cells (L5178Y/TK$^{+/-}$)	Forward mutation	–	–	Chun and Killeen 1989b
MMA	Rat heptocytes	Unscheduled DNA synthesis	No data	–	Chun and Killeen 1989d
DMA	Human peripheral lymphocytes	Mitogenesis inhibited	No data	–	Endo et al. 1992
DMA	Human lymphocytes	Sister chromatid exchange	No data	–	Rasmussen and Menzel 1997
DMA	Human alveolar (L-132) cells	Lung-specific DNA damage	No data	+	Kato et al. 1994
DMA	Human alveolar type II (L-132) cells	DNA single-strand breaks	+	+	Kawaguchi et al. 1996
DMA	Human diploid L-132 epithelial cells	DNA single-strand breaks	No data	+	Rin et al. 1995
DMA	Human alveolar type II (L-132) cells	DNA strand breaks	No data	+	Tezuka et al. 1993
DMA	Human embryonic cell line of type II alveolar epithelial cells (L-132)	DNA single-strand breaks and DNA-protein crosslinks	No data	+	Yamanaka et al. 1993
DMA	Human alveolar epithelial (L-132) cells	DNA single-strand breaks and DNA-protein crosslinks	No data	+	Yamanaka et al. 1995
DMA	Human pulmonary epithelial (L-132) cells	DNA single-strand breaks	No data	+	Yamanaka et al. 1997
DMA	Human umbilical cord fibroblasts	Chromosomal aberrations	No data	+	Oya-Ohta et al. 1996
DMA	Mouse lymphoma cells (L5178Y/TK^{+}/$^{-}$ -3.7.2C)	Chromosomal mutations	No data	+	Moore et al. 1997a
DMA	Chinese hamster lung and diploid cells (V79)	Mitotic arrest and tetraploid formation	No data	+	Endo et al. 1992

Table 3-10. Genotoxicity of Organic Arsenic

Chemical form	Species (test system)	End point	Results: With activation	Results: Without activation	Reference
DMA	Chinese hamster V79 cells	Chromosomal aberrations	No data	+	Ueda et al. 1997
DMA	Chinese hamster lung and diploid cells (V79)	Chromosomal aberrations	No data	+	Kitamura et al. 2002
DMA	Chinese hamster lung and diploid cells (V79)	Chromosomal aberrations	+	+	Kuroda et al. 2004
DMA	Chinese hamster V79 cells	Tetraploids and mitotic arrest	No data	+	Eguchi et al. 1997
MMA	Human umbilical cord fibroblasts	Chromosomal aberrations	No data	+	Oya-Ohta et al. 1996
MMA	Chinese hamster V79 cells	Tetraploids and mitotic arrest	No data	+	Eguchi et al. 1997
Roxarsone	*Drosophila melanogaster*	Sex linked recessive	No data	–	NTP 1989b
Roxarsone	Rat hepatocyte	DNA double-strand breaks	No data	+	Storer et al. 1996
Roxarsone	A31-1-13 clone of BALB/c-3T3 cells	Transformation response and mutagenicity	No data	–	Matthews et al. 1993
Roxarsone	Mouse lymphoma (L5178Y) cells	Trifluorothymidine resistance	No data	+	NTP 1989b
Eukaryotic organisms (*in vivo*):					
DMA	Rat (oral exposure)	DNA single-stand breaks in lung	No data	+	Yamanaka and Okada 1994
DMA	Mouse (oral exposure)	DNA strand breaks in tissues	No data	+	Yamanaka et al. 1989b
DMA	Mouse (oral exposure)	DNA single-stand breaks in lung	No data	+	Yamanaka et al. 1993
DMA	Mouse (oral exposure)	DNA single-strand breaks in lung	No data	–	Yamanaka et al. 1989a
DMA	Mouse (oral exposure)	DNA adduct formation	No data	+	Yamanaka et al. 2001
DMA	Mouse (injection)	Aneuploidy in bone marrow cells	No data	+	Kashiwada et al. 1998

– = negative result; + = positive result; DMA = dimethylarsinic acid; DNA = deoxyribonucleic acid; MMA = monomethylarsonic acid

3.4 TOXICOKINETICS

There is an extensive database on the toxicokinetics of inorganic arsenic. Most studies have been performed in animals, but there are a number of studies in humans as well. These studies reveal the following main points:

- Both arsenate and arsenite are well absorbed by both the oral and inhalation routes. Absorption by the dermal route has not been well characterized, but is low compared to the other routes. Inorganic arsenic in soil is absorbed to a lesser extent than solutions of arsenic salts.
- The rate of absorption of arsenic in highly insoluble forms (e.g., arsenic sulfide, lead arsenate) is much lower than that of more soluble forms via both oral and inhalation routes.
- Once absorbed, arsenites are oxidized to arsenates and methylated. This process may then be repeated to result in dimethylated arsenic metabolites.
- Distribution of arsenic in the rat is quite different from other animal species, suggesting that the rat is probably not an appropriate toxicokinetic model for distribution, metabolism, or excretion of arsenic by humans.
- The As(+3) form undergoes enzymic methylation primarily in the liver to form MMA and DMA. The rate and relative proportion of methylation production varies among species. The rate of methylation varies considerably among tissues.
- Most arsenic is promptly excreted in the urine as a mixture of As(+3), As(+5), MMA, and DMA; DMA is usually the primary form in the urine. Smaller amounts are excreted in feces. Some arsenic may remain bound to tissues, depending inversely on the rate and extent of methylation.

Less information is available for the organic arsenicals. It appears that both MMA and DMA are well absorbed, but are rapidly excreted in the urine and feces. MMA may be methylated to DMA, but neither MMA nor DMA are demethylated to yield inorganic arsenic.

A review of the evidence that supports these conclusions is presented below.

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

Inorganic Arsenicals. Since arsenic exists in air as particulate matter, absorption across the lung involves two processes: deposition of the particles onto the lung surface, and absorption of arsenic from the deposited material. In lung cancer patients exposed to arsenic in cigarette smoke, deposition was

estimated to be about 40% and absorption was 75–85% (Holland et al. 1959). Thus, overall absorption (expressed as a percentage of inhaled arsenic) was about 30–34%. In workers exposed to arsenic trioxide dusts in smelters, the amount of arsenic excreted in the urine (the main route of excretion; see Section 3.4.4) was about 40–60% of the estimated inhaled dose (Pinto et al. 1976; Vahter et al. 1986). Absorption of arsenic trioxide dusts and fumes (assessed by measurement of urinary metabolites) correlated with time weighted average arsenic air concentrations from personal breathing zone air samplers (Offergelt et al. 1992). Correlations were best immediately after a shift and just before the start of the next shift. Although the percent deposition was not measured in these cases, it seems likely that nearly all of the deposited arsenic was absorbed. This conclusion is supported by intratracheal instillation studies in rats and hamsters, where clearance of oxy compounds of arsenic (sodium arsenite, sodium arsenate, arsenic trioxide) from the lung was rapid and nearly complete (60–90% within 1 day) (Marafante and Vahter 1987; Rhoads and Sanders 1985). In contrast, arsenic sulfide and lead arsenate were cleared more slowly (Marafante and Vahter 1987), indicating that the rate of absorption may be lower if the inhaled arsenic is in a highly insoluble form. There are no data to suggest that absorption of inhaled arsenic in children differs from that in adults.

Organic Arsenicals. No studies were located regarding absorption of organic arsenicals in humans or animals after inhalation exposure. However, DMA instilled in the lungs of rats was absorbed very rapidly (half-time of 2.2 minutes) and nearly completely (at least 92%) (Stevens et al. 1977). This indicates that organic arsenicals are likely to be well absorbed by the inhalation route.

3.4.1.2 Oral Exposure

Inorganic Arsenicals. Several studies in humans indicate that arsenates and arsenites are well absorbed across the gastrointestinal tract. The most direct evidence is from a study that evaluated the 6-day elimination of arsenic in healthy humans who were given water from a high-arsenic sampling site (arsenic species not specified) and that reported approximately 95% absorption (Zheng et al. 2002). A similar absorption efficiency can be estimated from measurements of fecal excretion in humans given oral doses of arsenite, where <5% was recovered in the feces (Bettley and O'Shea 1975). This indicates absorption was at least 95%. These results are supported by studies in which urinary excretion in humans was found to account for 55–87% of daily oral intakes of arsenate or arsenite (Buchet et al. 1981b; Crecelius 1977; Kumana et al. 2002; Mappes 1977; Tam et al. 1979b). In contrast, ingestion of arsenic triselenide (As_2Se_3) did not lead to a measurable increase in urinary excretion (Mappes 1977), indicating that

gastrointestinal absorption may be much lower if highly insoluble forms of arsenic are ingested. There are no data to suggest that absorption of arsenic from the gut in children differs from that in adults.

These observations in humans are supported by a number of studies in animals. Fecal excretion of arsenates and arsenites ranged from 2 to 10% in monkeys and mice, with 70% or more appearing in urine (Charbonneau et al. 1978; Roberts et al. 2002; Vahter 1981; Vahter and Norin 1980). Oral absorption of [^{73}As] labeled sodium arsenate in mice was unaffected by dose (0.0005–5 mg/kg) as reflected in percentage of dose excreted in feces over 48 hours (Hughes et al. 1994). Absorption ranged from 82 to 89% at all doses. Gonzalez et al. (1995) found that the percentage of arsenate that was absorbed in rats decreased as the dose increased from 6 to 480 μg, suggesting saturable, zero-order absorption of arsenate in this species. Hamsters appear to absorb somewhat less than humans, monkeys, and mice, since fecal excretion usually ranges from 10 to 40% (Marafante and Vahter 1987; Marafante et al. 1987a; Yamauchi and Yamamura 1985). Rabbits also appear to absorb less arsenate than humans, monkeys, or mice after oral exposure (Freeman et al. 1993). After a gavage dose of 1.95 mg/kg sodium arsenate, 45% of the arsenate was recovered in feces in males and 52% in females. As in humans, when highly insoluble arsenic compounds are administered (arsenic trisulfide, lead arsenate), gastrointestinal absorption is reduced 20–30% (Marafante and Vahter 1987).

Bioavailability of arsenic was measured in rabbits ingesting doses of smelting soils that contained arsenic primarily in the form of sulfides (Freeman et al. 1993). Bioavailability was assessed by comparing the amounts of arsenic that was excreted after ingestion of the soil to that excreted after an intravenous dose of sodium arsenate. The bioavailability of the arsenic in the ingested soil was 24±3.2% and that of sodium arsenate in the gavage dose was 50±5.7%. Approximately 80% of the arsenic from ingested soil was eliminated in the feces compared with 50% of the soluble oral dose and 10% of the injected dose. In another study, rabbits dosed with sodium arsenite (0.8 mg As/kg) had 5 times greater blood arsenic concentrations than rabbits dosed with arsenic-containing soil (2.8 mg As/kg), suggesting a lower bioavailability of the arsenic in soil (Davis et al. 1992).

Studies of the bioavailability of arsenic suggest that absorption of arsenic in ingested dust or soil is likely to be considerably less than absorption of arsenic from ingested salts (Davis et al. 1992, 1996; EPA 1997g; Freeman et al. 1993, 1995; Pascoe et al. 1994; Roberts et al. 2002, 2007; Rodriguez et al. 1999). Oral absorption of arsenic in a group of three female Cynomolgus monkeys from a soluble salt, soil, and household dust was compared with absorption of an intravenous dose of sodium arsenate (Freeman et al. 1995). Mean absolute percentage bioavailability based on urine arsenic excretion was reported at

67.6±2.6% (gavage), 19.2±1.5% (oral dust), and 13.8±3.3% (oral soil). Mean absolute percentage bioavailability based on blood arsenic levels was reported at 91.3±12.4% (gavage), 9.8±4.3% (oral dust), and 10.9±5.2% (oral soil). The arsenic in the dust and soil was approximately 3.5–5-fold (based on levels in the urine) and 8–9-fold (based on levels in the blood) less bioavailable than arsenic in solution. Two other studies in monkeys reported relative bioavailability of arsenic in soil from a number of locations (electrical substation, wood preserving sites, pesticide sites, cattle-dip sites, volcanic soil, and mining sites) ranged from 5 to 31% (Roberts et al. 2002, 2007). A study in beagle dogs fed with soil containing As_2O_5 or treated with intravenous soluble arsenic found that compared to injection the bioavailability of arsenic from ingested soil was 8.3±2.0% (Groen et al. 1993). The bioavailability of arsenic in soil has been studied in juvenile swine that received daily oral doses of soil or sodium arsenate (in food or by gavage) for 15 days (EPA 1997g). The soils were obtained from various mining and smelting sites and contained, in addition to arsenic at concentrations of 100–300 μg/g, lead at concentrations of 3,000–14,000 μg/g. The arsenic doses ranged from 1 to 65.4 μg/kg/day. The fraction of the arsenic dose excreted in urine was measured on days 7 and 14 and the relative bioavailability of the soil-borne arsenic was estimated as the ratio of urinary excretion fractions, soil arsenic:sodium arsenate. The mean relative bioavailability of soil-borne arsenic ranged from 0 to 98% in soils from seven different sites (mean±SD, 45%±32). Estimates for relative bioavailability of arsenic in samples of smelter slag and mine tailings ranged from 7 to 51% (mean±SD, 35%±27). Rodriguez et al. (1999) used a similar approach to estimate the relative bioavailability of arsenic in mine and smelter wastes (soils and solid materials) in juvenile swine. Samples included iron slag deposits and calcine deposits and had arsenic concentrations that ranged from 330 to 17,500 μg/g. Relative bioavailability (waste:sodium arsenate) ranged from 3 to 43% for 13 samples (mean, 21%) and was higher in iron slag wastes (mean, 25%) than in calcine wastes (mean, 13%).

Bioavailability of arsenic from soil is reduced by low solubility and inaccessibility due to the presence of secondary reaction products or insoluble matrix components (Davis et al. 1992). This is supported by studies conducted with *in vitro* simulations of the gastric and/or intestinal fluids (Hamel et al. 1998; Pouschat and Zagury 2006; Rodriguez et al. 1999; Ruby et al. 1996, 1999; Williams et al. 1998). When soils containing arsenic are incubated in simulated gastrointestinal fluids, only a fraction of the arsenic becomes soluble. Estimates of the soluble, or bioaccessible, arsenic fraction have ranged from 3 to 50% for various soils and mining and smelter waste materials (Pouschat and Zagury 2006; Rodriguez et al. 1999; Ruby et al. 1996); these estimates are similar to *in vivo* estimates of the relative bioavailability of arsenic in these same materials (Ruby et al. 1999).

Organic Arsenicals. Based on urinary excretion studies in volunteers, it appears that both MMA and DMA are well absorbed (at least 75–85%) across the gastrointestinal tract (Buchet et al. 1981a; Marafante et al. 1987b). This is supported by studies in animals, where at least 75% absorption has been observed for DMA (Marafante et al. 1987b; Stevens et al. 1977; Vahter et al. 1984; Yamauchi and Yamamura 1984) and MMA (Hughes et al. 2005; Yamauchi et al. 1988). In mice, the relative bioavailability of MMA appears to be dose-dependent; 81% was absorbed following a single gavage dose of 0.4 mg MMA/kg/day compared to 60% following administration of 4 mg MMA/kg/day (Hughes et al. 2005).

3.4.1.3 Dermal Exposure

Inorganic Arsenicals. No quantitative studies were located on absorption of inorganic arsenicals in humans after dermal exposure. Percutaneous absorption of [^{73}As] as arsenic acid (H_3AsO_4) alone and mixed with soil has been measured in skin from cadavers (Wester et al. 1993). Labeled arsenic was applied to skin in diffusion cells and transit through the skin into receptor fluid measured. After 24 hours, 0.93% of the dose passed through the skin and 0.98% remained in the skin after washing. Absorption was lower with [^{73}As] mixed with soil: 0.43% passed through the skin over 24 hours and 0.33% remained in the skin after washing.

Dermal absorption of arsenic has been measured in Rhesus monkeys (Lowney et al. 2005; Wester et al. 1993). After 24 hours, 6.4% of [^{73}As] as arsenic acid was absorbed systemically, as was 4.5% of [^{73}As] mixed with soil (Wester et al. 1993). Similarly, 2.8% of soluble arsenic in water was detected in the urine 24 hours after exposure (Lowney et al. 2005). However, arsenic from soil was poorly absorbed; 0.12% was detected in the urine after 24 hours. Differences between the Wester et al. (1993) and Lowney et al. (2005) studies in terms of uptake from soil may be due to the differences in forms of arsenic in the soil. In the Wester et al. (1993) study, soil was mixed with radiolabelled arsenic acid in water; Lowney et al. (2005) used soil samples from a pesticide manufacturing facility that historically manufactured arsenical pesticides (the arsenic was primarily in the iron oxide and iron silicate mineral phases). Lowney et al. (2005) also measured urinary levels of arsenic following dermal application of CCA residues and found that the levels did not increase from background. Uptake of arsenic into blood or tissues was undetectable for up to 24 hours in rats whose tails were immersed in solutions of sodium arsenate for 1 hour. However, arsenic began to increase in blood, liver, and spleen over the next 5 days (Dutkiewicz 1977). The rate of uptake was estimated to be 1–33 $\mu g/cm^2$/hour. These findings suggest that dermal exposure leads initially to arsenic binding to skin, and that the bound arsenic may slowly be taken up into the blood, even after exposure ends.

Organic Arsenicals. No studies were located on absorption of organic arsenicals in humans or animals after dermal exposure.

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

Inorganic Arsenicals. No studies were located on the distribution of arsenic in humans or animals after inhalation exposure, but intratracheal administration of arsenic trioxide to rats resulted in distribution of arsenic to the liver, kidney, skeleton, gastrointestinal tract, and other tissues (Rhoads and Sanders 1985). This is consistent with data from oral and parenteral studies (below), which indicate that absorbed arsenic is distributed throughout the body.

Organic Arsenicals. No studies were located regarding the distribution of organic arsenicals in humans or animals after inhalation exposure. However, DMA administered to rats by the intratracheal route was distributed throughout the body (Stevens et al. 1977), suggesting that inhalation of organic arsenicals would also lead to widespread distribution.

3.4.2.2 Oral Exposure

Inorganic Arsenicals. Analysis of tissues taken at autopsy from people who were exposed to background levels of arsenic in food and water revealed that arsenic is present in all tissues of the body (Liebscher and Smith 1968). Most tissues had about the same concentration level (0.05–0.15 ppm), while levels in hair (0.65 ppm) and nails (0.36 ppm) were somewhat higher. This indicates that there is little tendency for arsenic to accumulate preferentially in any internal organs. However, exposure levels may not have been high enough to cause elevated levels in tissues. Arsenic exposure may have been low enough that the methylation process in the body resulted in limited accumulation in internal organs. Tissue analysis of organs taken from an individual following death from ingestion of 8 g of arsenic trioxide (about 3 g of arsenic) showed a much higher concentration of arsenic in liver (147 μg/g) than in kidney (27 μg/g) or muscle, heart, spleen, pancreas, lungs, or cerebellum (11–12 μg/g) (Benramdane et al. 1999a). Small amounts were also found in other parts of the brain (8 μg/g), skin (3 μg/g), and hemolyzed blood (0.4 μg/g). Many studies have been performed where arsenic levels in hair and nails have been measured and correlations with exposure analyzed. Some of these studies are discussed in Section 3.8, Biomarkers of Exposure.

Inorganic arsenic passes easily through the placenta. High levels of arsenic were found in the liver, kidney, and brain during autopsy of an infant prematurely born to a young mother who had ingested inorganic arsenic at week 30 of gestation (Lugo et al. 1969). Arsenic was detected in human breast milk at concentrations of 0.00013–0.00082 ppm in a World Health Organization study (Somogyi and Beck 1993). Arsenic concentrations were 0.0001–0.0044 ppm in human milk sampled from 88 mothers on the Faroe Islands whose diets were predominantly seafood (Grandjean et al. 1995). Exposures to arsenic from the seafood diet in this population was most likely to organic "fish arsenic." In a population of Andean women exposed to high concentrations (about 200 ppb) of inorganic arsenic in drinking water, concentrations of arsenic in breast milk ranged from about 0.0008 to 0.008 ppm (Concha et al. 1998b).

Studies in mice and hamsters given oral doses of arsenate or arsenite have found elevated levels of arsenic in all tissues examined (Hughes et al. 2003; Vahter and Norin 1980; Yamauchi and Yamamura 1985), including the placenta and fetus of pregnant females (Hood et al. 1987, 1988). Inorganic arsenic crosses the placental barrier and selectively accumulates in the neuroepithelium of the developing animal embryo (Hanlon and Ferm 1977; Lindgren et al. 1984). In mice, radiolabel from orally administered 74-As was widely distributed to all tissues, with the highest levels in skin, kidney, and liver (Hughes et al. 2003). No obvious differences between As(+3) and As(+5) were found, although residual levels after 24 hours tended to be higher for As(+3) than As(+5) (Vahter and Norin 1980). However, *in vitro* studies have found that the cellular uptake of As(+3) was higher than that of As(+5) (Bertolero et al. 1987; Dopp et al. 2004); in mouse cells, the difference was 4-fold (Bertolero et al. 1987). In hamsters, increases in tissue levels were noted after oral treatment with As(+3) for most tissues (hair, kidney, liver, lung, skin, muscle), with the largest increases in liver and lung (Yamauchi and Yamamura 1985). Liver and kidney arsenic concentrations increased with dose in dogs fed arsenite in the diet for 6 months (Neiger and Osweiler 1992). A study examining the speciation of arsenic following a single dose exposure to sodium arsenate to mice (Kenyon et al. 2005) found that the levels of inorganic arsenic and DMA were similar in the blood, liver, and kidney; much lower levels of MMA were found in these tissues. The concentration of DMA in the lungs exceeded inorganic arsenic and the levels of inorganic arsenic and MMA were similar; the DMA concentration was about 6 times higher than that of inorganic arsenic.

Inorganic arsenic crosses the placental barrier and selectively accumulates in the neuroepithelium of the developing animal embryo (Hanlon and Ferm 1977; Lindgren et al. 1984). Following maternal exposure to arsenite or arsenate throughout gestation and lactation, inorganic arsenic and DMA were detected in the

newborn mouse brains (Jin et al. 2006). The levels of inorganic arsenic in the brain were similar to those in the newborn livers; however, the levels of DMA in the brain were about twice as high as in the liver.

Organic Arsenicals. No studies were located on the distribution of organic arsenicals in humans following oral exposure. Studies in animals found MMA and DMA distributed to all tissues after acute oral doses (Hughes et al. 2005; Stevens et al. 1977; Vahter et al. 1984; Yamauchi and Yamamura 1984; Yamauchi et al. 1988). In mice, MMA is rapidly distributed throughout the body with peak tissue concentrations occurring between 0.25 and 4 hours after administration of a single gavage dose of 0.4 or 4 mg MMA/kg (Hughes et al. 2005). The peak levels of MMA in the bladder, kidneys, and lungs were higher than blood, with the highest levels occurring in the bladder. The terminal half-lives of MMA were 4.2–4.9 hours in the liver, lung, and blood, 9.0 hours in the urinary bladder, and 15.9 hours in the kidney in mice dosed with 0.4 mg MMA/kg; similar half-lives were measured in the 4.0 mg MMA/kg mice. Two hours after dosing, most of the methylated arsenic in the tissues was in the form of MMA. In rats exposed to 100 mg/kg DMA in the diet for 72 days, high levels of arsenic was detected in the blood (Lu et al. 2004a). The arsenic was primarily found in the erythrocyte; the concentration in the erythrocyte was 150 times higher than the arsenic concentration in the plasma.

3.4.2.3 Dermal Exposure

No studies were located regarding distribution of inorganic or organic arsenicals in humans or animals after dermal exposure.

3.4.2.4 Other Routes of Exposure

Inorganic Arsenicals. Studies in mice, rabbits, and monkeys injected intravenously with solutions of arsenite or arsenate confirm that arsenic is widely distributed throughout the body (Lindgren et al. 1982; Marafante and Vahter 1986; Vahter and Marafante 1983; Vahter et al. 1982). Shortly after exposure, the concentration of arsenic tends to be somewhat higher in liver, kidney, lung, and gastrointestinal epithelium (Hughes et al. 2000; Lindgren et al. 1982; Vahter and Marafante 1983; Vahter et al. 1982), but levels tend to equilibrate over time. Arsenate shows a tendency to deposit in skeletal tissue that is not shared by arsenite (Lindgren et al. 1982, 1984), presumably because arsenate is an analog of phosphate.

The distribution of arsenic in the rat is quite different from other animal species. Following intramuscular injection of carrier-free radio-arsenate in rats, most of the injected arsenic became bound to hemoglobin in red blood cells, and very little reached other tissues (Lanz et al. 1950). However, similar experiments

in dogs, mice, guinea pigs, rabbits, and chicks found very little uptake of arsenic into the blood in these species (cats gave intermediate results).

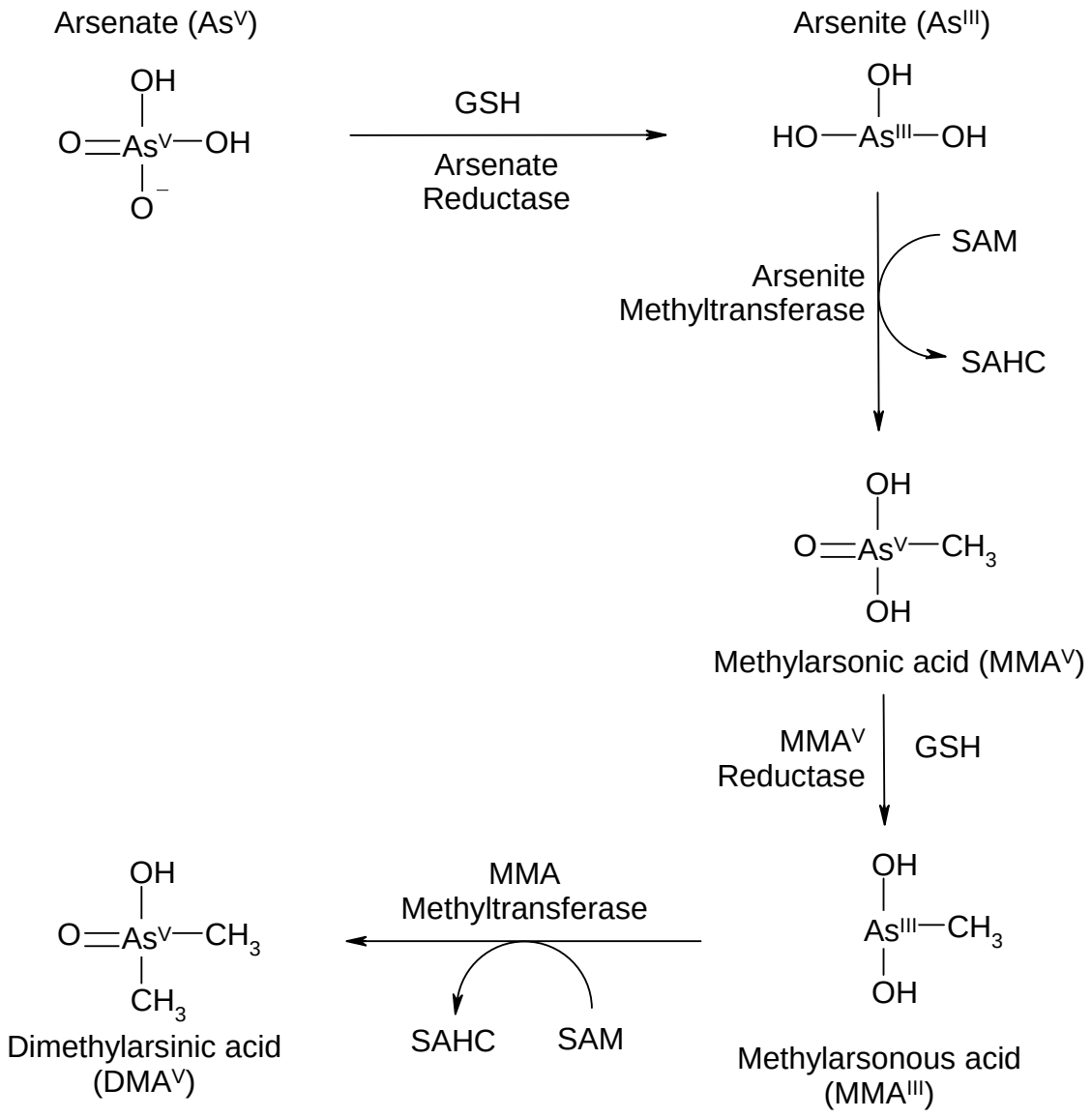
Organic Arsenicals. Following intravenous administration of DMA in mice, DMA is rapidly distributed throughout the body (Hughes et al. 2000). In the blood, the DMA was initially detected in the plasma, but fairly rapidly equilibrated between the plasma and erythrocytes. Blood, plasma, erythrocyte, liver, and kidney distribution and elimination of DMA did not differ in groups of mice administered 1.11 or 111 mg DMA/kg. However, a significant difference in DMA elimination from the lungs was observed; the elimination half-time increased from 91 minutes in the 1.11 mg DMA/kg group to 6,930 minutes in the 111 mg DMA/kg group.

3.4.3 Metabolism

Inorganic Arsenicals. The metabolism of inorganic arsenic has been extensively studied in humans and animals, and is diagrammed in Figure 3-7. Two basic processes are involved: (1) reduction/oxidation reactions that interconvert As(III) and As(V), and (2) methylation reactions, which convert arsenite to MMA and DMA. The resulting series of reactions results in the reduction of inorganic arsenate to arsenite (if necessary), methylation to MMA(V), reduction to MMA(III), and methylation to DMA(V). These processes appear to be similar whether exposure is by the inhalation, oral, or parenteral route. The human body has the ability to change inorganic arsenic to organic forms (i.e., by methylation) that are more readily excreted in urine. In addition, inorganic arsenic is also directly excreted in the urine. It is estimated that by means of these two processes, >75% of the absorbed arsenic dose is excreted in the urine (Marcus and Rispin 1988), although this may vary with the dose and exposure duration. This mechanism is thought to have an upper-dose limit which, when overwhelmed, results in a higher incidence of arsenic toxicity. This is supported by a case report of an individual who died 3 days after ingesting 8 g of arsenic trioxide (about 3 g of arsenic) (Benramdane et al. 1999a). Only 20% of the total arsenic in all tissues analyzed was methylated (14% MMA, 6% DMA), while 78% remained as arsenite and 2% as arsenate.

The majority of the evidence characterizing the metabolic pathways of arsenic is derived from analysis of urinary excretion products. Exposure of humans to either arsenates or arsenites results in increased levels of inorganic As(+3), inorganic As(+5), MMA, and DMA in urine (Aposhian et al. 2000a, 2000b; Buchet et al. 1981a, 1981b; Concha et al. 1998a, 1998b; Crecelius 1977; Kurttio et al. 1998; Lovell and Farmer

Figure 3-7. Inorganic Arsenic Biotransformation Pathway



SAHC = S-adenosylhomocysteine; SAM = S-adenosylmethionine

Source: adapted from Aposhian et al. 2000b

1985; Smith et al. 1977; Tam et al. 1979b; Vahter 1986). Similar results are obtained from studies in mice (Vahter 1981; Vahter and Envall 1983), hamsters (Hirata et al. 1988; Marafante and Vahter 1987; Takahashi et al. 1988), and rabbits (Maiorino and Aposhian 1985; Marafante et al. 1985; Vahter and Marafante 1983). Historically, little distinction was made between MMA(V) and MMA(III) in the urine in most studies, and the assumption was that the majority of MMA in the urine was MMA(V); however, Aposhian et al. (2000a, 2000b) demonstrated that the methylated arsenic atom may be in either valance state.

The relative proportions of As(+3), As(+5), MMA, and DMA in urine can vary depending upon the chemical administered, time after exposure, route of exposure, dose level, and exposed species. In general, however, DMA is the principal metabolite following long-term exposure, with lower levels of inorganic arsenic [As(+3) and As(+5)] and MMA. In humans, the relative proportions are usually about 40–75% DMA, 20–25% inorganic arsenic, and 15–25% MMA (Buchet et al. 1981a; Hopenhayn et al. 2003b; Loffredo et al. 2003; Mandal et al. 2001; Smith et al. 1977; Tam et al. 1979b; Tokunaga et al. 2002; Vahter 1986). With relatively constant exposure levels, these metabolic proportions remain similar over time (Concha et al. 2002), and appear to be similar among family members (Chung et al. 2002). One study of groups of women and children in two villages in Argentina showed that children ingesting large amounts of arsenic in their drinking water (200 μg/L) excreted about 49% inorganic arsenic and 47% DMA (Concha et al. 1998b). This compared to 32% inorganic arsenic and 66% DMA for the women in the study. This may indicate that metabolism of arsenic in children is less efficient than in adults. The rabbit has a ratio of metabolites similar to human adults (Maiorino and Aposhian 1985), suggesting that this may be a good animal model for toxicokinetics in humans. Mice may also be a good human toxicokinetic model based on the similarity of arsenic metabolism and deposition (Vahter et al. 2002). In contrast, the guinea pig, marmoset, and tamarin monkey do not methylate inorganic arsenic (Healy et al. 1998; Vahter and Marafante 1985; Vahter et al. 1982; Zakharyan et al. 1996); thus, they may be poor models for humans.

Reduction of arsenate to arsenite can be mediated by glutathione (Menzel et al. 1994). Scott et al. (1993) showed that glutathione forms complexes with both arsenate and arsenite *in vitro*, and that glutathione is oxidized (and arsenate reduced) in the glutathione-arsenate reaction. Studies *in vitro* indicate that the substrate for methylation is As(+3), and that As(+5) is not methylated unless it is first reduced to As(+3) (Buchet and Lauwerys 1985, 1988; Lerman et al. 1983). The main site of methylation appears to be the liver, where the methylation process is mediated by enzymes that utilize S-adenosylmethionine as cosubstrate (Buchet and Lauwerys 1985, 1988). Under normal conditions, the availability of methyl

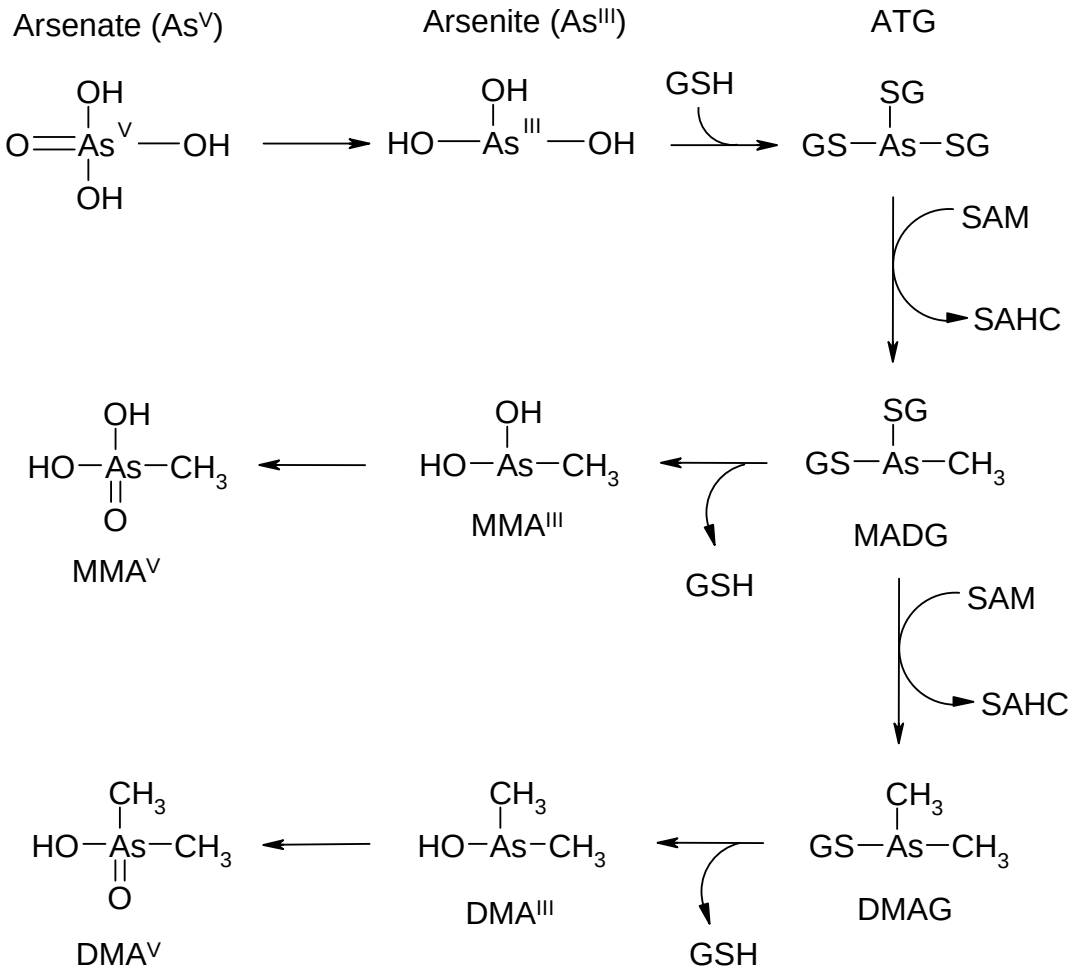
donors (e.g., methionine, choline, cysteine) does not appear to be rate limiting in methylating capacity, either in humans (Buchet et al. 1982) or in animals (Buchet and Lauwerys 1987; Buchet et al. 1981a). However, severe dietary restriction of methyl donor intake can result in significant decreases in methylating capacity (Buchet and Lauwerys 1987; Vahter and Marafante 1987).

Arsenic methyltransferase and MMA methyltransferase activities have been purified to homogeneity from cytosol of rabbit liver (Zakharyan et al. 1995), Rhesus monkey liver (Zakharyan et al. 1996), and rat liver (Thomas et al. 2004). It appears that a single protein catalyzes both activities. This activity transfers a methyl group from S-adenosylmethionine to As(+3) yielding MMA, which is then further methylated to DMA. Reduced glutathione is probably a co-factor *in vivo*, but other thiols can substitute *in vitro* (L-cysteine, dithiothreitol). The substrate saturation concentration for rabbit arsenite methyltransferase is 50 μM, for MMA methyltransferase it is 1,000 μM. The purified activity is specific for arsenite and MMA; selenite, selenate, selenide, and catechols do not serve as substrates. Thomas et al. (2004) reported cloning the gene for an S-adenosylmethionine-dependent methyltransferase from rat liver cytosol that catalyzes the conversion of arsenic to methylated and dimethylated species. It bears a high similarity to translations of *cyt19* genes in both the mouse and the human; both this gene and protein are now termed arsenic (+3 oxidation state) methyltransferase (AS3MT).

Studies in mice indicate that exposure to arsenic does not induce arsenic methylation activity (Healy et al. 1998). Mice receiving up to 0.87 mg As/kg/day as sodium arsenate in drinking water for 91 days had the same arsenic methylating activity as unexposed controls. Specific activities were highest in testis (1.45 U/mg) followed by kidney (0.70 U/mg), liver (0.40 U/mg), and lung (0.20 U/mg). None were affected by arsenic exposure.

An alternative biotransformation pathway (Figure 3-8) has recently been proposed for arsenic (Hayakawa et al. 2005) based on the nonenzymatic formation of glutathione complexes with arsenite resulting in the formation of arsenic triglutathione. The arsenic triglutathione is subsequently methylated by AS3MT to form monomethyl arsenic glutathione. At low glutathione levels (1 mM), the monomethyl arsenic glutathione is hydrolyzed to form MMA(III). At high glutathione levels (5 mM), the monomethyl arsenic glutathione is methylated to dimethylarsinic glutathione by AS3MT. Dimethylarsinic glutathione is quickly hydrolyzed to form DMA(III) (Hayakawa et al. 2005; Thomas et al. 2007). In the classical inorganic arsenic biotransformation pathway (Figure 3-7), MMA(V) is converted to the more toxic MMA(III); in contrast, in the alternative pathway, MMA(III) is converted to the less toxic MMA(V).

Figure 3-8. Alternative Inorganic Arsenic Biotransformation Pathway



ATG = arsenic triglutathione; DMAG = dimethylarsinic glutathione; GSH = glutathione; MADG = monomethyl arsenic glutathione; SAHC = S-adenosylhomocysteine; SAM = S-adenosylmethionine

Source: Hayakawa et al. 2005; Thomas et al. 2007

Since methylation tends to result in lower tissue retention of inorganic arsenic (Marafante and Vahter 1984, 1986; Marafante et al. 1985; Vahter and Marafante 1987), the methylation process is usually viewed as a detoxification mechanism. However, several studies showing an elevated toxicity of MMA(III) relative even to As(III) in cultured human liver cells (Petrick et al. 2000, 2001) have called this assumption into question. Because methylation is an enzymic process, an important issue is the dose of arsenic that saturates the methylation capacity of an organism, resulting in a possible increased level of the more toxic As(III) in tissues, or whether or not such a dose exists. Limited data from studies in humans suggest that methylation may begin to become limiting at doses of about 0.2–1 mg/day (0.003–0.015 mg/kg/day) (Buchet et al. 1981b; Marcus and Rispin 1988). However, these observations are relatively uncertain since they are based on data from only a few subjects, and the pattern of urinary excretion products in humans who ingested high (near lethal) oral doses or were exposed to elevated levels in the workplace is not much different from that in the general population (Lovell and Farmer 1985; Vahter 1986). Furthermore, the nutrient intakes reported by Engel and Receveur (1993) were sufficient to accommodate the body stores of methyl groups needed for arsenic biomethylation. At the highest arsenic level reported in the endemic area, the biomethylation process required only a few percent of the total daily methyl intake (Mushak and Crocetti 1995). Thus, the dose rate at which methylation capacity becomes saturated cannot be precisely defined with current data.

Organic Arsenicals. With the exception of arsenosugars, which may undergo extensive metabolism, organic arsenicals appear to undergo little metabolism. Humans who ingested a dose of MMA converted a small amount (about 13%) to DMA (Buchet et al. 1981a). Similarly, in mice and hamsters, DMA and MMA are primarily excreted unchanged in the urine (Hughes et al. 2005; Marafante et al. 1987b; Vahter et al. 1984). In mice, a small percentage of MMA is methylated to DMA and some is further methylated to trimethylarsine oxide (TMAO) (Hughes et al. 2005). In contrast, administration of MMA(III) to mice resulted in the excretion of mostly DMA(V) and smaller amounts of MMA(V), MMA(III), and DMA(III) (Hughes et al. 2005). As with MMA, only a small percentage (<10%) of the DMA is methylated to TMAO (Hughes et al. 2005; Marafante et al. 1987b; Yamauchi and Yamamura 1984; Yamauchi et al. 1988).

MMA and DMA are more extensively methylated in rats compared to other animal species. After 1 week of exposure to 100 mg As/kg/day as MMA in drinking water, rats excreted 50.6% of the total arsenic in urine as MMA, 19.0% as DMA, 6.9% as TMAO, and 0.4% as tetramethylarsonium (Yoshida et al. 1998). In contrast, mice exposed to a single dose of 40 mg As/kg as MMA excreted 89.6% of the dose as MMA, 6.2% as DMA, and 1.9% as TMAO (Hughes et al. 2005). Similarly, 24 hours after administration of a

single oral dose of 50 mg As/kg as MMA in hamsters, 26.9% was excreted in urine as MMA, 1.43% as DMA, and 0.07% as trimethylarsenic compound (Yamauchi et al. 1988). As with MMA, oral exposure of mice and hamsters to DMA results in most of the dose being excreted in the urine in the form of DMA (or DMA complex) (Marafante et al. 1987b); in rats, the levels of DMA and TMAO are about equal (Yoshida et al. 1998).

The available data suggest that the methylarsenates are not demethylated to inorganic arsenic either in humans (Buchet et al. 1981a; Marafante et al. 1987b) or in animals (rats and hamsters) (Stevens et al. 1977; Yamauchi and Yamamura 1984; Yoshida et al. 2001).

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

Inorganic Arsenicals. As noted previously (see Section 3.4.1.1), urinary excretion of arsenic appears to account for 30–60% of the inhaled dose (Holland et al. 1959; Pinto et al. 1976; Vahter et al. 1986). Since the deposition fraction usually ranges from about 30 to 60% for most respirable particles (EPA 1989b), this suggests that nearly all arsenic that is deposited in the lung is excreted in the urine. The time course of excretion in humans exposed by inhalation has not been thoroughly investigated, but urinary arsenic levels in workers in a smelter rose within hours after they came to work on Monday and then fell over the weekend (Vahter et al. 1986). This implies that excretion is fairly rapid, and this is supported by intratracheal studies in rats (Rhoads and Sanders 1985) and hamsters (Marafante and Vahter 1987), where whole-body clearance of administered arsenate or arsenite occurred with a half-time of 1 day or less. However, the study in rats (Rhoads and Sanders 1985) found that the clearance of arsenic trioxide was biphasic, with 95% cleared with a half-time of 29 minutes and the remaining arsenic cleared with a half-time of 75 days. For sodium arsenate and sodium arsenite, <0.1% of the dose was retained in the lung 3 days after exposure of hamsters; 1.3% of the arsenic trisulfide dose was retained after 3 days (Marafante and Vahter 1987). The Marafante and Vahter (1987) study suggested that lung clearance was influenced by compound solubility. The primary forms of arsenic found in the urine of inhalation-exposed humans are DMA and MMA, with inorganic arsenic comprising <25% of the total urinary arsenic (Apostoli et al. 1999).

Organic Arsenicals. No studies were located regarding the excretion of organic arsenicals by humans or animals after inhalation exposure. However, rats that were given a single intratracheal dose of DMA

excreted about 60% in the urine and about 8% in the feces within 24 hours (Stevens et al. 1977). This indicates that organic arsenicals are likely to be promptly excreted after inhalation exposure.

3.4.4.2 Oral Exposure

Inorganic Arsenicals. Direct measurements of arsenic excretion in humans who ingested known amounts of arsenite or arsenate indicate that very little is excreted in the feces (Bettley and O'Shea 1975), and that 45–85% is excreted in urine within 1–3 days (Apostoli et al. 1999; Buchet et al. 1981a; Crecelius 1977; Mappes 1977; Tam et al. 1979b). At low exposure levels, urinary arsenic levels generally increase linearly with increasing arsenic intake (Calderon et al. 1999). During lactation, a very small percent of ingested arsenic may also be excreted in the breast milk (Concha et al. 1998a). A similar pattern of urinary and fecal excretion is observed in hamsters (Marafante and Vahter 1987; Yamauchi and Yamamura 1985) and mice (Vahter and Norin 1980); this pattern is typically modeled as a biphasic process (e.g., Hughes et al. 2003). Generally, whole body clearance is fairly rapid, with half-times of 40–60 hours in humans (Buchet et al. 1981b; Mappes 1977). Clearance is even more rapid in mice and hamsters, with 90% removed in 2 days (Hughes et al. 2003; Marafante and Vahter 1987; Vahter 1981; Vahter and Norin 1980).

A study in pregnant women exposed to elevated levels of inorganic arsenic in drinking water found that most of the ingested arsenic was excreted in the urine as DMA (79–85%), with smaller amounts excreted as inorganic arsenic (8–16%) or MMA (5–6%) (Christian et al. 2006). Similarly, in mice, arsenate is primarily excreted in the urine as DMA, with lesser amounts of inorganic arsenic and MMA (Kenyon et al. 2005). Following a single oral dose of 10 μmol/kg sodium arsenate, 78.4% was excreted as DMA, 20.2% as inorganic arsenic, and 1.45% as MMA; at a 10-fold higher dose, the ratio of DMA to inorganic arsenic decreased (57.7% DMA, 39.8% inorganic arsenic, and 2.59% MMA).

Arsenic is also excreted in the bile via the formation of two arsenic-glutathione complexes (arsenic triglutathione and methylarsenic diglutathione) (Kala et al. 2000). In rats administered 5.0 mg/kg sodium arsenite, equal amounts of arsenic triglutathione and methylarsenic diglutathione were found in the bile 18–20 minutes after exposure. At a lower arsenic dose (0.5 mg/kg), only methylarsenic diglutathione was found. As discussed in Section 3.4.4.4, biliary excretion of arsenic has also been detected in mice, hamsters, guinea pigs, and rabbits following parenteral exposure (Csanaky and Gregus 2002).

Organic Arsenicals. Studies in humans indicate that ingested MMA and DMA are excreted mainly in the urine (75–85%), and this occurs mostly within 1 day (Buchet et al. 1981a; Marafante et al. 1987b). This is supported by studies in rats, mice, and hamsters, although in animals, excretion is more evenly distributed between urine and feces (Hughes et al. 2005; Marafante et al. 1987b; Stevens et al. 1977; Yamauchi and Yamamura 1984; Yamauchi et al. 1988). In mice administered 40 mg As/kg as DMA, 56.4% was excreted in the urine as DMA, 7.7% as a DMA complex, and 3.5% as TMAO during a 48-hour period after dosing; in the feces, 24.3% was DMA and 4.9% as DMA complex (Marafante et al. 1987b). In hamsters, 38.7% was DMA, 11.2% as DMA complex, and 6.4% as TMAO in the urine; in the feces, 37.3% as DMA and 4.9% as DMA complex. As with DMA, most MMA is excreted in the urine and feces as parent compound. In the urine of mice administered 0.4 mg As/kg as MMA, 98.2% of the urinary arsenicals was in the form of MMA(V) and 1.8% as MMA(III) (Hughes et al. 2005); at a 10-fold higher dose, 89.6% was excreted as MMA(V), 1.2% as MMA(III), 6.2% as DMA(V), 1.1% as DMA(III), and 1.9% as TMAO. As discussed previously, exposure of rats to MMA or DMA results in the excretion of a higher percentage of metabolites. After 1 week exposure to MMA, 50.6% of the dose was excreted as MMA, 19.0% as DMA, and 6.9% of TMAO (Yoshida et al. 1998). A 1-week exposure to DMA, 44.9% was excreted as DMA in the urine and 40.0% as TMAO (Yoshida et al. 1998). A longer-term exposure to DMA (>7 months) resulted in a higher percentage of the amount of parent compound excreted; 56–65% as DMA and 23–35% as TMAO (Li et al. 1998; Wanibuchi et al. 1996; Yoshida et al. 1998).

In mice and hamsters, DMA and MMA are rapidly cleared from the body (Hughes et al. 2005; Marafante et al. 1987b; Vahter et al. 1984). In mice, 85% of the initial oral dose of DMA was eliminated from the body with a half-life of 2.5 hours (Vahter et al. 1984). In contrast to the mouse data, 45% on the initial DMA dose to rats was eliminated with a half-time of 13 hours and the remaining 55% had an elimination half-time of 50 days (Vahter et al. 1984).

3.4.4.3 Dermal Exposure

Inorganic Arsenicals. No studies were located regarding excretion of inorganic arsenicals in humans or animals following dermal exposure. In rats, arsenic absorbed through the tail was excreted approximately equally in urine and feces, similar to the excretion pattern following oral exposure (Dutkiewicz 1977).

Organic Arsenicals. No studies were located regarding excretion of organic arsenicals in humans or animals following dermal exposure.

3.4.4.4 Other Routes of Exposure

Inorganic Arsenicals. Excretion of arsenate and arsenite following parenteral exposure of animals is similar to that seen following oral exposure. In rabbits and mice, urinary excretion within 8 hours usually accounts for about 50–80% of the dose (Maehashi and Murata 1986; Maiorino and Aposhian 1985; Vahter and Marafante 1983). Somewhat lower levels (30–40%) are excreted in the urine of marmoset monkeys (Vahter and Marafante 1985; Vahter et al. 1982), probably because of the absence of methylation in this species. Whole-body clearance studies in mice indicate that arsenate is over 65% removed within 24 hours, while arsenite is about 86% removed at 24 hours (Lindgren et al. 1982). A relatively small proportion of an injected dose of arsenic V (10% for rats, 4% for mice, and <2% for hamsters, guinea pigs, and rabbits) was found to be excreted into the bile within the first 2 hours postinjection (Csanaky and Gregus 2002). Following arsenic III injection, a much greater percentage (92% for guinea pigs and 75% for rats) of the arsenic was found in the bile in the first 2 hours after administration (Csanaky and Gregus 2002). Similarly, approximately 40% of an intravenous dose of sodium arsenite was excreted into the bile of rats, most of it occurring during the first hour after exposure (Kala et al. 2000). Kala et al. (2000) determined that the biliary transport of arsenic was dependent on the formation of arsenic-glutathione complexes, which were transported out of hepatocytes by multidrug resistance associated protein 2 (MRP2/cMOAT); most of the arsenic in bile was in the form of arsenic triglutathione or methylarsenic diglutathione.

3.4.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen and

Krishnan 1994; Andersen et al. 1987). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

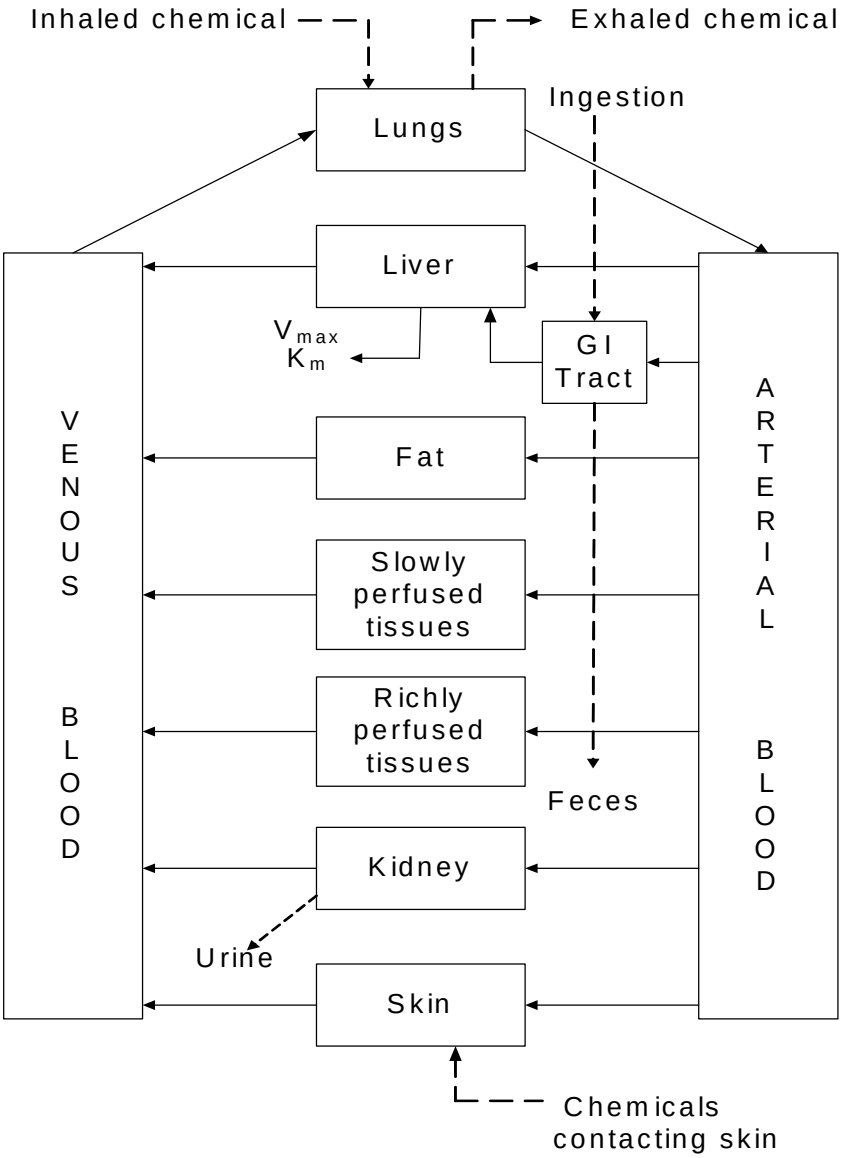
The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parameterization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) are adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-9 shows a conceptualized representation of a PBPK model.

If PBPK models for arsenic exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

Figure 3-9. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

Source: adapted from Krishnan and Andersen 1994

Several PBPK models have been developed for inorganic arsenic; the Mann, Yu, and Menzel models are discussed below. A joint research effort between the Chemical Industry Institute of Toxicology (CIIT) Centers for Health Research, EPA, ENVIRON International, and the Electric Power Research Institute (EPRI) is underway to develop a biologically based dose response model of carcinogenicity. Part of this effort involves refining the existing PBPK models (Clewell et al. 2007).

3.4.5.1 Summary of PBPK Models

The Mann model (Gentry et al. 2004; Mann et al. 1996a, 1996b), Yu model (Yu 1998a, 1998b; Yu 1999a, 1999b), and Menzel model (Menzel et al. 1994) are the PBPK models for arsenic currently available. The Mann model simulates the absorption, distribution, metabolism, elimination, and excretion of As(+3), As(+5), MMA, and DMA after oral and inhalation exposure in mice, hamsters, rabbits, and humans. The Yu model simulates the absorption, distribution, metabolism, elimination, and excretion of As(+3), As(+5), MMA, and DMA after oral exposure to inorganic arsenic in mice, rats, or humans. The Menzel model is a preliminary model that predicts internal organ burden of arsenic during specific oral exposures, simulating the metabolism, distribution to organs and binding to organs in mice, rats, and humans.

3.4.5.2 Arsenic PBPK Model Comparison

The Mann model is a well-derived model, consisting of multiple compartments and metabolic processes, and modeling four chemical forms of arsenic (two organic and two inorganic), which has been validated using experimental data. The Yu model has more compartments than the Mann model, also models metabolism and fate of four forms of arsenic, and has likewise been validated using experimental data. The Menzel model is still preliminary and has not been validated.

3.4.5.3 Discussion of Models

The Mann Model

Risk assessment. The Mann model was not used for risk assessment.

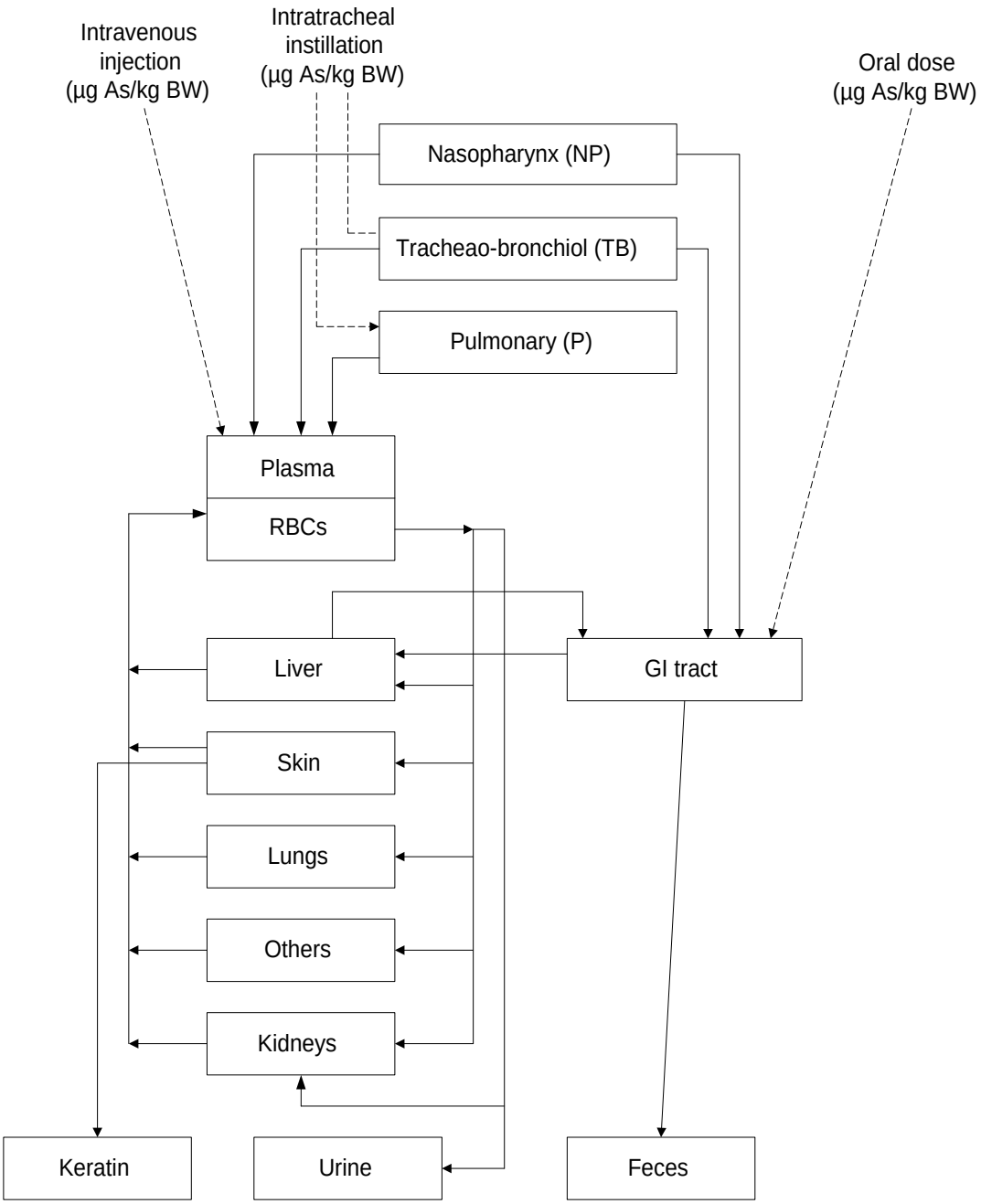
Description of the model. The Mann model was initially developed to simulate oral, intratracheal, and intravenous exposure to arsenic in rabbits and hamsters (Mann et al. 1996a). In a companion paper, the model was expanded to include inhalation exposure and extrapolated and applied to humans (Mann et al. 1996b). A subsequent paper further expanded the model to include mice (Gentry et al. 2004).

The model consists of six tissue compartments: blood, liver, kidneys, lungs, skin, and other tissues. The blood compartment is divided into plasma and red blood cell subcompartments, considered to be at equilibrium. Three routes of exposure are considered in the model. Oral exposure is considered to enter the liver from the gastrointestinal tract via first-order kinetics. Intratracheal exposure results in deposition into the pulmonary and tracheo-bronchial regions of the respiratory tract. Uptake into blood from the pulmonary region is considered to be via first order kinetics into plasma, uptake from the tracheo-bronchial region is by both transfer into plasma and transport into the gastrointestinal tract. Intravenous injection results in a single bolus dose into the plasma compartment.

Metabolism in the model consists of oxidation/reduction and two methylation reactions. The oxidation/reduction of inorganic arsenic was modeled as a first order process in the plasma, with reduction also included in the kidneys. Methylation of As(+3) was modeled as a two-step process occurring in the liver according to Michaelis-Menton kinetics.

Most physiological parameters were derived by scaling to body weight. In cases where parameters were not available (absorption rates, tissue affinity, biotransformation), estimates were obtained by fitting. This was done by duplicating the initial conditions of published experiments in the model, varying the unknown parameters and comparing the results of the simulation to the reported results. Tissue affinity constants were estimated using reported arsenic levels in tissues at various times after exposure. Metabolic rate constants and absorption rate constants were estimated using data for excretion of arsenic metabolites in urine and feces. Figure 3-10 shows the animal model and Tables 3-11, 3-12, 3-13, and 3-14 provide the parameters used in the animal model. The human model is similar to the animal models with adjustments for body weight and absorption and metabolic rates. A naso-pharynx compartment is included in the human model, which was not present in the animal models. Penetration and deposition in the respiratory tract are based on the log-normal particle size distribution of the aerosol. Metabolic and absorption rate constants were fitted using experimental data on urinary excretion of arsenic following a single oral dose of As(+3) (Buchet et al. 1981a) or As(+5) (Tam et al. 1979b) in volunteers. The lung absorption rate constant was obtained by fitting the total urinary excretion of arsenic as predicted with the model to experimental data obtained from occupational exposure to arsenic trioxide (Offergelt et al. 1992). Figure 3-11 shows the human model, and Tables 3-15 and 3-16 provide the data and constants used in the human model.

Figure 3-10. Parameters Used in the Mann PBPK Model for Animals



Source: Mann et al. 1996b

Table 3-11. Parameters Used in the Mann PBPK Model for Animals

Physiological parameter	Rabbit (body weight=3.5 kg)	Hamster (body weight=0.100 kg)
Blood volume (mL)	253	7.0
Organ weight (g)		
Liver	121	4.8
Kidneys	25	1.2
Lungs	31	1.0
Skin	420	17.1
Organ volume (mL)		
Others	2,386	62.0
Lumen volume (mL)		
Stomach	15	0.5
Small intestine	20	0.6
Blood flow (mL/minute)		
Cardiac output	556	38.3
Liver, hepatic	25	1.2
Liver, splanchic	98	6.0
Kidneys	100	7.0
Lungs	13	0.7
Skin	38	2.6
Others	282	20.8
Clearance (mL/minute)		
Glomerular Filtration Rate	10	0.6
Small intestine length (cm)	180	56.0
Total capillary surface area (cm^2)	93,835	2,681.0

PBPK = physiologically based pharmacokinetic

Source: Mann et al. 1996a

Table 3-12. Tissue Affinity Constants (K_{ij}) Obtained for the Mann PBPK Model for Animals by Fitting for Rabbits and Hamsters

	K_{ij} (unitless)			
Tissue (*i*)	As(V)	As(III)	MMA	DMA
Liver	1	200	10	1
Kidneys	40	20	100	5
Lungs	1	1	1	20
Skin	1	60	50	1
Others	10	40	1	1

DMA = dimethylarsinic acid; MMA = monomethylarsonic acid; PBPK = physiologically based pharmacokinetic

Source: Mann et al. 1996a

Table 3-13. Metabolic Rate Constants for the Mann PBPK Model for Animals Obtained by Fitting for Rabbits and Hamsters

Oxidation/reduction	First order	Rabbit	Hamster
Reduction	(1/hour)	3,000.00	100.00
Oxidation	(1/hour)	6,000.00	400.00
Kidney reduction	(1/hour)	30.00	1.00
Methylation	Michaelis–Menten		
1st step	$K_{M_{MMA}}$ (μmol/mL)	0.05	0.12
	$V_{MAX_{MMA}}$ (μmol/mL-hour)	4.00	0.12
2nd step	$K_{M_{DMA}}$ (μmol/mL)	0.90	0.08
	$V_{MAX_{DMA}}$ (μmol/mL-hour)	1.50	0.12

DMA = dimethylarsinic acid; MMA = monomethylarsonic acid; PBPK = physiologically based pharmacokinetic

Source: Mann et al. 1996a

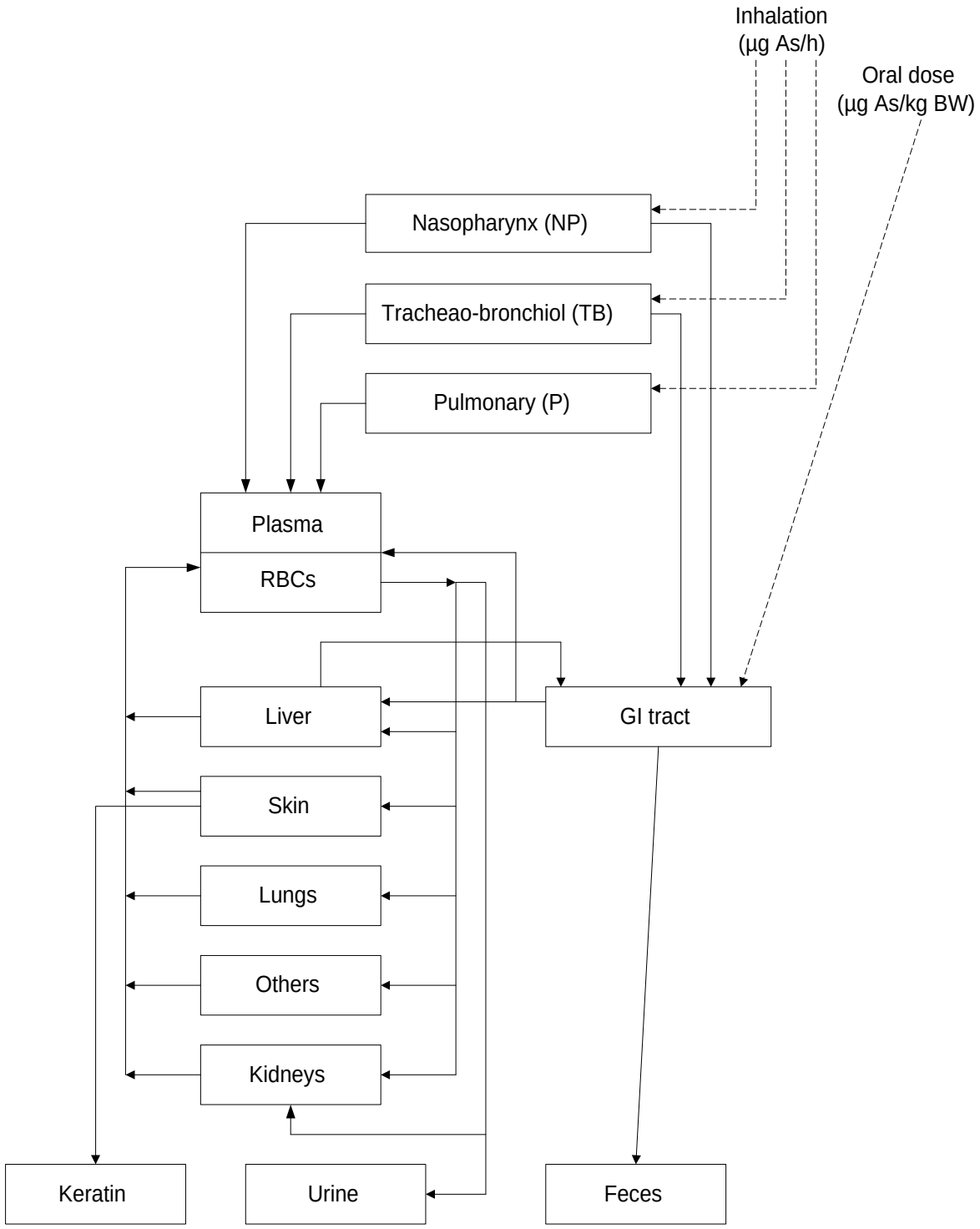
Table 3-14. Fitted Gastrointestinal Tract and Lung Absorption Half-time for the Hamster for the Mann PBPK Model

	Absorption, half-time (hour)	
Arsenic compound	Gastrointestinal tract	Lung
As(V)		
$Na_3(AsO_4)$	0.08	12
$Pb_3(AsO_4)$	0.39	690
As_2O_5	0.28	—
As(III)		
$NaAsO_2$	0.08	12
As_2S_3	0.48	12
As_2O_3	0.02	—
DMA	0.09	—

DMA = dimethylarsinic acid; PBPK = physiologically based pharmacokinetic

Source: Mann et al. 1996a

Figure 3-11. Parameters Used in the Mann PBPK Model for Humans



Source: Mann et al. 1996b

Table 3-15. Physiological Data Used in the Mann PBPK Model for Humans

Physiological parameter	Organ	Units	Human (body weight=70 kg)
Blood volume		mL	5,222
Organ weight	Liver	g	1,856
	Kidneys	g	314
	Lungs	g	584
	Skin	g	6,225
	Others	g	55,277
Lumen volume	Stomach	mL	274
	Small intestine	mL	393
Blood flow	Cardiac output	L/minute	5.29
	Liver, hepatic	L/minute	0.32
	Liver, splanchic	L/minute	1.02
	Kidneys	L/minute	0.95
	Lungs	L/minute	0.16
	Skin	L/minute	0.35
	Others	L/minute	2.49
Creatinine			
Male		g/day	1.7
Female		g/day	1.0
Clearance			
Glomerular filtration rate		mL/minute	156
Small intestine length		cm	481
Nasopharynx area		cm^2	177
Tracheobronchial area		cm^2	5,036
Pulmonary area		cm^2	712,471
Total capillary surface area		cm^2	$1,877 \times 10^6$

DMA = dimethylarsinic acid; MMA = monomethylarsonic acid; PBPK = physiologically based pharmacokinetic

Source: Mann et al. 1996b

Table 3-16. Tissue Affinity Constants (K_{ij}) Obtained by Fitting the Mann PBPK Animal Model for Use with Humans

	K_{ij} (unitless)			
Tissue (i)	As(V)	As(III)	MMA	DMA
Liver	1	200	10	1
Kidneys	40	20	100	5
Lungs	1	1	1	20
Skin	1	60	50	1
Red blood cells	0.2	1.5	0.2	0.2
Others	10	40	1	1

DMA = dimethylarsinic acid; MMA = monomethylarsonic acid; PBPK = physiologically based pharmacokinetic

Source: Mann et al. 1996b

Validation of the model. The model was generally successful in describing the disposition of an intravenous dose of sodium arsenate in rabbits over a 24-hour period (Marafante et al. 1985). Discrepancies included a 6–7-fold overestimation of levels in skin at 24 hours and underestimation of As(+5) in plasma in the hour following injection. A statistical assessment of how well the model fit the empirical data was not presented. In hamsters, the model was also generally predictive of oral and intratracheal exposures (Marafante and Vahter 1987). Generally, predictions were better for the exposures to As(+5) than for those to As(+3).

The human model was validated using data from studies of repeated oral intake of sodium arsenite in volunteers (Buchet et al. 1981b), occupational exposure to arsenic trioxide and elemental arsenic (Vahter et al. 1986), and community exposure to As(+5) via drinking water (Harrington et al. 1978; Valentine et al. 1979). Simulations were generally in good agreement with the experimental data.

The predictions of tissue distribution, metabolism, and elimination of arsenic compounds from the mouse model were compared with experimental data, and showed generally good agreement. The model tended to overpredict the concentration of organic arsenicals in the lungs, and to a lesser extent in the kidneys and liver, while for inorganic arsenic, the model overpredicted the levels of arsenic (V) present in the urine of acutely-exposed mice.

Target tissues. Levels in skin were not well predicted by this model in animals. Results for the lung were not presented, except for the mouse model, which tended to overpredict lung levels. The human model was only used to predict urinary metabolites.

Species extrapolation. Species extrapolation was not attempted in this model. However, tissue affinities derived for the rabbit and hamster models were used in the human model.

Interroute extrapolation. Interroute extrapolation was not attempted in this model.

The Menzel Model

Risk assessment. The Menzel model was not used for risk assessment.

Description of the model. The Menzel model was developed to simulate oral exposure to arsenic from drinking water and food. Inhalation of arsenic in the particulate phase or as arsine gas is not considered. The chemical species in drinking water is assumed to be As(+5).

The model consists of two sets of compartments: those in which the pools of arsenic are not influenced by blood perfusion, and those in which blood perfusion does determine arsenic burden. The former set of compartments includes the gut, feces, hair, bladder, and urine. The latter set of compartments included lung, liver, fat, skin, kidney, and other tissues. Oral exposure is considered to enter the liver from the gastrointestinal tract.

The model followed that of Andersen and coworkers (Andersen et al. 1987; Ramsey and Andersen 1984). Data from mice were used to test predictions of absorption. Excretion is considered to be rapid and complete into the urine, with no reabsorption from the kidney. Fecal arsenic content accounts for unabsorbed arsenic excreted in the bile, and complex arsenic species from food. Metabolism includes reduction by glutathione and methylation. Arsenic accumulation in the skin, hair and nails was included by assuming that arsenic binds irreversibly to protein sulfide groups in hair and nails.

Validation of the model. The model was preliminary and has not been validated.

Target tissues. Target tissues have not yet been modeled.

Species extrapolation. Species extrapolation was not attempted in this model.

Interroute extrapolation. Interroute extrapolation was not attempted in this model.

The Yu Model

Risk assessment. The Yu model was not used for risk assessment.

Description of the model. The Yu model was developed to simulate oral exposure to arsenic in mice and rats (Yu 1998a, 1998b), and was later adapted for oral exposures in humans (Yu 1999a, 1999b). Inhalation of arsenic in the particulate phase or as arsine gas is not considered. As(+3), As(+5), MMA, and DMA were all considered in the model, though the movements of MMA and DMA were not considered.

The model consists of eight tissue compartments: intestine, skin, muscle, fat, kidney, liver, lung, and vessel-rich group (VRG, e.g., brain); in the human model, the VRG and kidney compartments were combined. Only oral exposure was considered. Absorption is based on absorption to the stomach, which then passes the arsenic to the gastrointestinal tract. From the gastrointestinal tract, arsenic is either transferred to the blood or excreted in the feces.

The physiological parameters for the model were obtained from published values in the literature. Tissue/blood partition coefficients were based on the postmortem blood and tissue concentrations from a fatal human poisoning case study (Saady et al. 1989). Tissue volumes and blood flow rates were based on published values from a number of sources (EPA 1988e; Reitz et al. 1990). Absorption and excretion rate constants were based on experimental observations of blood concentrations and urinary and fecal excretion following oral administration of inorganic arsenic (Odanaka et al. 1980; Pomroy et al. 1980). Metabolic rate constants for the methylation and dimethylation of inorganic arsenic were also based on experimental observations (Buchet et al. 1981a; Crecelius 1977). Figure 3-12 shows the model and Table 3-17 provides the parameters used for each species.

Validation of the model. The model was generally successful at predicting the urinary excretion 48 hours after administration of 5 mg/kg inorganic arsenic in both rats and mice. After 48 hours, the observed/predicted ratios associated with excreted doses ranged from 0.78 to 1.11 for the mouse and from 0.85 to 0.93 for the rat. However, the model overpredicted the amount of inorganic arsenic found in the feces of mice at 24 and 48 hours, and overpredicted the amount of DMA formed by exposed mice at 48 hours. In rats, the model overestimated the urinary and fecal excretion of inorganic arsenic at 24 hours postexposure, though at 48 hours, measured values all fell within the predicted ranges. The human model was also generally successful at predicting the urinary excretion of arsenic compounds following oral exposure, based on results of controlled human exposure studies (Buchet et al. 1981a; Vahter 1983). In general, however, the model underpredicts excretion at early time points and overpredicts at later time points, with 24 hours being the time at which its predictive capabilities agreed most strongly with available data.

The ability of the model to predict tissue burdens was not compared to actual data for any species.

Figure 3-12. Parameters Used in the Yu PBPK Model for Animals

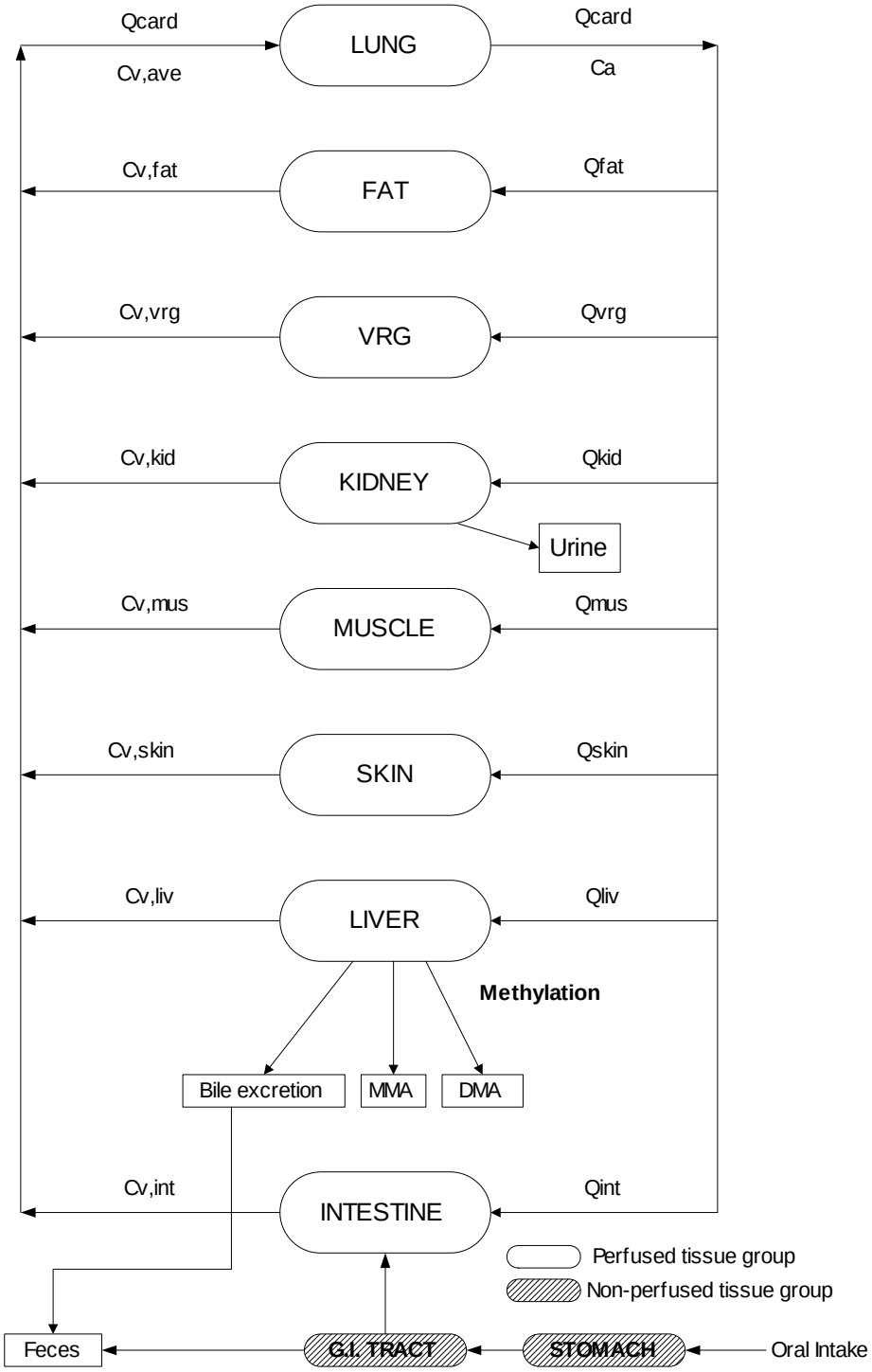


Table 3-17. Parameters Used in the Yu PBPK Model

	Mouse	Rat	Human
Partition coefficients			(As^{III}/As^{V}/MMA/DMA)
Intestine	6.0	6.0	2.8/2.8/1.2/1.4
Skin	5.0	5.0	2.5/2.5/1.25/1.25
VRG	6.0	6.0	Combined with kidney
Muscle	5.0	10.0	2.6/2.6/1.8/2.8
Fat	—	0.5	0.3/0.3/0.3/0.3
Kidney	8.5	7.5	4.15/4.15/1.8/2.075
Liver	10.0	10.0	5.5/5.3/2.35/2.65
Lung	4.0	4.0	4.15/4.15/1.8/2.075
Blood flow rate (mL/hour)			
Intestine	100	528	1,810
Skin	7.68	37.8	130
VRG	157	960	N/A
Muscle	153	1,260	25,850
Fat	—	253.2	6,467
Kidney	255	255	45,240
Liver	255	1,260	32,320
Lung	N/R	N/R	129,000
Tissue volume (mL)			
Intestine	1.94	6.9	558
Skin	1.83	15.4	606
VRG	0.81	23.0	N/A
Muscle	19.9	162	6,989
Fat	—	14.5	2,328
Kidney	0.484	1.63	248
Liver	1.67	5.82	422
Lung	0.124	1.0	400
Metabolism constants			
$Vmax_{(MMA)}$ (μmol/hour)	0.45	0.15	11.25
$Vmax_{(DMA)}$ (μmol/hour)	0.375	0.06	22.25
$Km_{(MMA)}$ (μmol/hour)	1.0	0.2	0.01
$Km_{(DMA)}$ (μmol/hour)	0.2	0.2	0.01

Table 3-17. Parameters Used in the Yu PBPK Model

	Mouse	Rat	Human
First-order rate constants			(As^{III}/As^{V}/MMA/DMA)
K_{SI} ($hour^{-1}$)	0.3	0.3	-/1.2/-/-
K_{AI} ($hour^{-1}$)	1.5	3.6	-/1.2/-/-
K_{fecal} ($hour^{-1}$)	0.33	0.048	-/0.0012/0.0/0.0
$K_{urinary}$ ($hour^{-1}$)	1.32	0.9	0.05/0.075/0.07/0.04
$K_{biliary}$ ($hour^{-1}$)	0.33	0.3	-/0.018/-/-

DMA = dimethylarsinic acid; MMA = monomethylarsonic acid; N/A = not applicable; N/R = not reported

Source: Yu 1998a, 1998b, 1999a, 1999b

Target tissues. Model predictions of tissue burdens were not compared to actual data. The model accurately predicted, with a few exceptions, the urinary and fecal excretion of inorganic arsenic and its metabolites in rats, mice, and humans.

Species extrapolation. Species extrapolation beyond rats and mice was not attempted using this model. The human model has not been compared to, or linked with, either of the rodent models.

Interroute extrapolation. Interroute extrapolation was not attempted using this model.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Arsenic absorption depends on its chemical form. In humans, As(+3), As(+5), MMA, and DMA are orally absorbed ≥75%. Arsenic is also easily absorbed via inhalation. Absorption appears to be by passive diffusion in humans and mice, although there is evidence (Gonzalez et al. 1995) for a saturable carrier-mediated cellular transport process for arsenate in rats (for review, see Rosen 2002). Dermal absorption appears to be much less than by the oral or inhalation routes. Bioavailability of arsenic from soil appears to be lower via the oral route than it is for sodium salts of arsenic. Arsenic in soil may form water insoluble compounds (e.g., sulfides), which are poorly absorbed.

Arsenic and its metabolites distribute to all organs in the body; preferential distribution has not been observed in human tissues at autopsy or in experiments with animal species other than rat (in which arsenic is concentrated in red blood cells). Since the liver is a major site for the methylation of inorganic arsenic, a “first-pass” effect is possible after gastrointestinal absorption; however, this has not been investigated in animal models.

Arsenic and its metabolites are largely excreted via the renal route. This excretion mechanism is not likely to be saturated within the dose range expected from human exposure. Excretion can also occur via feces after oral exposure; a minor excretion pathway is nails and hair. The methylation of inorganic arsenic is the major metabolism pathway. The proportion of metabolites recovered in urine (As(+3), As(+5), MMA, DMA) are roughly consistent in humans regardless of the exposure scenario. However, interindividual variation is great enough that it cannot be determined if capacity limitation may occur in some individuals.

The manifestation of arsenic toxicity depends on dose and duration of exposure. Single oral doses in the range of 2 mg As/kg and higher have caused death in humans. Doses as low as 0.05 mg As/kg/day over longer periods (weeks to months) have caused gastrointestinal, hematological, hepatic, dermal, and neurological effects. These effects appear to be a result of direct cytotoxicity. Long-term exposure (years) to drinking water at levels as low as 0.001 mg As/kg/day have been associated with skin diseases and skin, bladder, kidney, and liver cancer. Long-term inhalation exposure to arsenic has also been associated with lung cancer at air levels as low as 0.05–0.07 mg/m^3. It is not clear at this time why long-term toxicity is different between the oral and inhalation routes, given that arsenic is easily absorbed into the systemic circulation by both routes.

Studies in mice and rats have shown that arsenic compounds induce metallothionein, a metal-binding protein thought to detoxify cadmium and other heavy metals, *in vivo* (Albores et al. 1992; Hochadel and Waalkes 1997; Kreppel et al. 1993; Maitani et al. 1987a). The potency of arsenic compounds in inducing metallothionein parallels their toxicity (i.e., As(+3) > As(+5) > MMA > DMA). For cadmium, it is thought that metallothionein binds the metal, making it biologically inactive. For arsenic, however, only a small percentage of the administered arsenic is actually bound to metallothionein (Albores et al. 1992; Kreppel et al. 1994; Maitani et al. 1987a). *In vitro* studies have shown that affinity of arsenic for metallothionein is much lower than that of cadmium or zinc (Waalkes et al. 1984). It has been proposed that metallothionein might protect against arsenic toxicity by acting as an antioxidant against oxidative injury produced by arsenic (NRC 1999).

3.5.2 Mechanisms of Toxicity

Mechanisms of arsenic-induced toxicity and carcinogenicity have not been clearly identified. However, recent efforts to elucidate mechanisms of arsenic toxicity and carcinogenicity have resulted in numerous *in vitro* and *in vivo* reports. Whereas these mechanistic studies typically employed relatively high arsenic exposure levels, some of the most recent studies were performed using more environmentally-relevant exposure levels. Due to the extremely large amount of mechanistic data for arsenic, it is not feasible to include all pertinent primary studies that address issues concerning proposed mechanisms of arsenic toxicity and carcinogenicity. Therefore, the following discussion of mechanisms of arsenic toxicity represents a summary of information from several recent review articles (Chen et al. 2004, 2005; Florea et al. 2005; Hughes 2002; Kitchin 2001; Lantz and Hays 2006; Navas-Acien et al. 2005; Rossman 2003; Roy and Saha 2002; Thomas et al. 2007; Vahter 2002).

It is becoming increasingly evident that the toxicity and carcinogenicity of arsenic is likely to be closely associated with metabolic processes. Absorbed pentavalent arsenic (AsV) is rapidly reduced to trivalent arsenic (AsIII) at least partially in the blood. Much of the formed AsIII is distributed to tissues and taken up by cells (particularly hepatocytes). Many cell types appear to accumulate AsIII more rapidly than AsV. Because AsIII (as arsenite) is known to be more highly toxic than AsV (as arsenate), the reduction step may be considered bioactivation rather than detoxification. Glutathione appears to play a role in the reduction of AsV to AsIII, which is required prior to methylation. Methylation of arsenic ultimately forms relatively less toxic MMA and DMA; this process is accomplished by alternating between the reduction of AsV to AsIII and the addition of a methyl group; S-adenosylmethionine is considered to be the source of the methyl group. Both MMA and DMA are less reactive with tissue constituents than inorganic arsenic and both are readily excreted in the urine. The methylation process appears to include multiple intermediates, some of which are more reactive than inorganic arsenic. For example, reactive trivalent metabolites, MMAIII and DMAIII, have been detected in the urine of human subjects chronically exposed to arsenic in drinking water, and *in vitro* studies have demonstrated MMAIII to be more toxic than arsenite or arsenate to human hepatocytes, epidermal keratinocytes, and bronchial epithelial cells. Additional *in vitro* studies have demonstrated genotoxic and DNA damaging properties of both MMAIII and DMAIII.

AsV (as arsenate) has been demonstrated to: (1) replace phosphate in glucose-6-phosphate and 6-phosphogluconate *in vitro*, (2) replace phosphate in the sodium pump and the anion exchange transport system of human red blood cells, (3) diminish the *in vitro* formation of adenosine-t′-triphosphate (ATP) by replacing phosphate in enzymatic reactions, and (4) deplete ATP in some cellular systems, but not in human erythrocytes. However, it is becoming more apparent that the major source of arsenic toxicity and carcinogenicity is related to its reduction to arsenite.

AsIII (as arsenite) is known to react with thiol-containing molecules such as glutathione and cysteine *in vitro*. Methylated trivalent arsenics such as MMAIII are potent inhibitors of glutathione reductase and thioredoxin reductase. It has been suggested that binding of arsenite and methylated trivalent arsenicals to critical thiol groups could lead to the inhibition of essential biochemical reactions, alteration of cellular redox status, and eventual cytotoxicity. Binding of MMAIII and DMAIII to protein has also been demonstrated *in vitro*. Arsenite inhibits pyruvate dehydrogenase (PDH), a complex that oxidizes pyruvate to acetyl-CoA, a precursor to intermediates of the citric acid cycle that provides reducing equivalents to the electron transport system for ATP production. This property may explain the depletion of carbohydrates in arsenite-treated rats.

Evidence that arsenic may induce alterations in nitric oxide metabolism and endothelial function includes findings that persons exposed to high levels of arsenic in drinking water had decreased serum and urine concentrations of nitric oxide metabolites, which was reversed upon intervention with drinking water containing lower levels of arsenic. Urinary arsenic levels have been inversely associated with nitric oxide production in activated monocytes. Arsenite concentrations of 1–25 μM inhibited endothelial nitric oxide synthase activity and resulting decreased cell growth in human endothelial cells, although lower concentrations up-regulated the expression of constitutive nitric oxide synthase 3, which might serve as an explanation for observed arsenic-induced cell growth and angiogenesis.

Although epidemiological studies demonstrate the carcinogenicity of arsenic in humans, early animal cancer bioassays failed to demonstrate a carcinogenic effect following lifetime exposure to inorganic arsenic. However, more recent focus has resulted in the development of animal models that exhibit carcinogenic activity in skin, urinary bladder, liver, and lung, tissues implicated in arsenic-induced cancer in humans. This concordance in target sites among animal models and humans indicates that common mechanisms of action may be applicable to humans and laboratory animals.

Several modes of action have been proposed to explain, at least in part, the carcinogenicity of arsenic. It is likely that multiple mechanisms are involved, some of which may relate to noncancer effects as well.

Oxidative Stress. Mechanistic studies of arsenic toxicity have suggested a role of the generation of reactive oxygen species in the toxicity of inorganic arsenic. Results of both *in vivo* and *in vitro* studies of arsenic-exposed humans and animals suggest the possible involvement of increased lipid peroxidation, superoxide production, hydroxyl radical formation, blood nonprotein sulfhydrals, and/or oxidant-induced DNA damage. Reduction of cellular oxidant defense by treatment with glutathione-depleting agents results in an increased sensitivity of cells to arsenic toxicity. Support for mechanisms of toxicity that involves arsenic-induced oxidative stress includes findings that inhaled arsenic can predispose the lung to oxidative damage, chronic low-dose arsenic alters genes and proteins that are associated with oxidative stress and inflammation, and major transcriptional regulators of altered genes are redox sensitive.

Genotoxicity. Collectively, *in vitro* and *in vivo* genotoxicity assays have demonstrated that arsenics cause single strand breaks, formation of apurinic/apyrimidinic sites, DNA base and oxidative base damage, DNA-protein crosslinks, chromosomal aberrations, aneuploidy, sister chromatid exchanges, and micronuclei. Chromosomal aberrations, characterized by chromatid gaps, breaks and fragmentation,

endoreduplication, and chromosomal breaks, are dose-dependent and arsenite is more potent than arsenate. Both MMAIII and DMAIII are directly genotoxic and are many times more potent than arsenite at inducing DNA damage. Inorganic arsenic can potentiate the mutagenicity observed with other chemicals, although arsenic itself does not appear to induce point mutations. Arsenic-induced genotoxicity may involve oxidants or free radical species.

Altered Growth Factors→Cell Proliferation→Promotion of Carcinogenesis. Increased concentrations of growth factors can lead to cell proliferation and eventual promotion of carcinogenesis. Arsenic-induced cell death can also lead to compensatory cell regeneration and carcinogenesis. Altered growth factors, cell proliferation, and promotion of carcinogenesis have all been demonstrated in one or more systems exposed to arsenics. Altered growth factors and mitogenesis were noted in human keratinocytes. Cell death was observed in human hepatocytes and rat bladder epithelium. Cell proliferation was demonstrated in human keratinocytes and intact human skin and rodent bladder cells. Promotion of carcinogenesis was noted in rat bladder, kidney, liver, and thyroid, and mouse skin and lung.

Additional Mechanisms of Toxicity Data. Inorganic arsenic exposure has been shown to modify the expression of a variety of genes related to cell growth and defense, including the tumor suppressor gene p53, as well as to alter the binding of nuclear transcription factors. Carcinogenic effects of arsenic may result from a cocarcinogenic effect. Whereas arsenic exposure alone did not elicit skin tumors in mice, co-exposure to arsenic and ultraviolet light resulted in skin tumors that were greater in number and larger in size than those produced by ultraviolet light alone. Arsenate and arsenite enhanced the amplification of a gene that codes for the enzyme dihydrofolate reductase, arsenate being more potent than arsenite. Furthermore, inhibition of DNA repair has been demonstrated in arsenic-treated cells.

3.5.3 Animal-to-Human Extrapolations

The usefulness of animal models for toxicity studies with arsenic is significantly limited by two major factors. First and most importantly, no animal model exists for the health effect of greatest concern for human exposure: carcinogenicity in skin and other organs after oral exposure. Second, the pattern of metabolism in humans (significant excretion of the methylated forms of arsenic) is unlike that of most other mammalian species (the mouse and rabbit may be exceptions). The ratios of inorganic to organic arsenic excreted also vary between species. The rat sequesters arsenic in its erythrocytes and is not a suitable model for human toxicity.

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Thomas and Colborn (1992), was also used in 1996 when Congress mandated the EPA to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), and in 1998, the EDSTAC completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997h). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

There is little evidence to suggest that arsenic functions as an endocrine disruptor. An association has been demonstrated between exposure to arsenic in drinking water and increased incidence of diabetes mellitus (Rahman et al. 1998; Tsai et al. 1999; Tseng et al. 2000; Wang et al. 2003), although dose-response relationships are not available and the mechanism of action for this response has not been characterized. Studies by Waalkes and coworkers (Waalkes et al. 2006a, 2006b) have suggested that in

mice, arsenic may interact with estrogens to enhance production of female urogenital cancers and male hepatocellular cancer following exposure to arsenic *in utero*. The mechanism by which this might happen has not been elucidated. No other relevant data were located in humans or animals. Data on general effects of arsenic compounds on the endocrine system are presented in Sections 3.2.1.2 and 3.2.2.2 above.

In vitro studies provide suggestive evidence that arsenic may act as an endocrine disruptor. Studies by Bodwell et al. (2004, 2006) and Davey et al. (2007) demonstrate that arsenic can alter gene regulation of steroid hormone receptors for glucocorticoids, mineralocorticoids, progesterone, and estrogen.

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6, Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life, and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water, and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek

1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

Arsenic has been recognized as a human toxicant for many centuries, and the symptoms of acute poisoning are well known. Children who are exposed to high levels of arsenic exhibit symptoms similar to those seen in adults, including respiratory, cardiovascular, dermal, and neurological effects, and vomiting if the arsenic is ingested (Borgoño et al. 1980; Foy et al. 1992; Kersjes et al. 1987; Muzi et al. 2001; Rosenberg 1974; Zaldívar 1974; Zaldívar and Guillier 1977). Arterial thickening of the pancreas was observed in five children who died in Chile after chronic exposure to arsenic (Rosenberg 1974). Foy et al. (1992) described systemic effects of chronic arsenic exposure in children in a village near a tin and tungsten mining operation in Thailand. The arsenic concentration in water samples from 35 shallow wells averaged 0.82 mg As/L (range, 0.02–2.7 mg As/L). Piped water (available in some homes) had a concentration of 0.07 mg As/L. A survey of skin manifestations of arsenic poisonings was conducted in the autumn of 1987. The case reports of four children were presented. All of the children had hyperkeratosis and hyperpigmentation of the extremities, including tibia, palms, and soles. In addition, one child had developed weakness 3 years previously and had anorexia and a chronic cough for 1 year. She had been held back twice in school as a slow learner. On examination, she had a runny nose and

weakness of her wrist joints. The liver was about 4 finger-breadths below the right costal margin with a sharp but tender edge. Blood arsenic levels ranged from 0.087 to 0.46 μg/mL and the arsenic level in hair ranged from 14.4 to 20 μg/g. The authors concluded that the finding of typical skin manifestations of chronic arsenic poisoning suggests that it may take a considerably shorter period of time to develop these manifestations than previously thought. However, it is not known what effect co-exposure to tin and tungsten might have had on skin manifestations in these children. Exposure to high arsenic levels during gestation and/or during early childhood also was associated with significant increases in SMRs for lung cancer and bronchiectasis during adulthood in a study of residents in a city in Chile with high arsenic levels in the drinking water (near 0.9 mg/L) during several years (Smith et al. 2006).

As previously mentioned in Sections 3.2.1.4 and 3.2.2.4, exposure of children to arsenic also has been associated with neurological deficits in children. Studies by Wasserman et al. (2004, 2007) of 6- and 10-year-old children from Bangladesh reported small but significant decreases in some tests of cognitive function associated with levels of arsenic in the water ≥0.05 mg/L. A study of pre-school age children in West Bengal, India, reported an association between current urinary arsenic concentrations, but not long-term water arsenic, and small decrements in intellectual tests (von Ehrenstein et al. 2007). Similar results were reported in a study of children in Taiwan (Tsai et al. 2003) and in China (Wang et al. 2007). Neurological effects have also been associated with elevated levels of arsenic in the air. For example, Bencko et al. (1977) reported that children of approximately 10 years of age living near a power plant burning coal of high arsenic content showed significant hearing losses (increased threshold) compared to a control group of children living outside the polluted area (Bencko et al. 1977). Also, in a study of Mexican children, Calderón et al. (2001) reported that children living near a smelter complex had poor performance on tests evaluating verbal IQ than children who lived farther from the smelter. Thus, the limited data available suggest that exposure of children to inorganic arsenic may result in detrimental effects on neurobehavioral parameters.

Wulff et al. (1996) conducted a retrospective study of a cohort of children born between 1961 and 1990 in the municipality of Skelleftea, Sweden, where a smelter released arsenic and other pollutants including lead, copper, cadmium, and sulfur dioxide. Childhood cancer incidences among children born in the vicinity of the smelter (i.e., within 20 km) and distant from the smelter (>20 km) were compared with expected incidences based on Swedish national statistics. There appeared to be an increased risk of childhood cancer (all types combined) among children born in the vicinity of the smelter (SIR=195, 95% CI=88–300, based on 13 cases observed and 6.7 expected), but the increase was not statistically significant, and in any event, the role of arsenic in any finding from this study is confounded by the

presence of other metals. The number of cases (n=42) was very close to the expected number (n=41.8) among children born distant from the smelter. Similar results were reported in a study by Moore et al. (2002), which did not find increased incidence ratios for all childhood cancers or for childhood leukemias in children from an area of Nevada with high arsenic exposures.

Inorganic arsenic has been characterized as a developmental toxicant. It is known to cross the placental barrier and selectively accumulate in the neuroepithelium of the developing animal embryo (Hanlon and Ferm 1977; Lindgren et al. 1984). Studies in animals have also revealed that various fetal malformations occur after embryonic exposure to arsenic *in vitro*; neural tube defects are the predominant and consistent malformation in these studies (Chaineau et al. 1990; Mirkes and Cornel 1992; Morrissey and Mottet 1983; Mottet and Ferm 1983; Tabacova et al. 1996; Willhite and Ferm 1984; Wlodarczyk et al. 1996). *In vivo* studies have shown that high doses of ingested arsenic can produce developmental effects (fetal mortality, skeletal defects), but generally only at maternally toxic doses (Baxley et al. 1981; Holson et al. 1999, 2000; Hood and Harrison 1982; Hood et al. 1978; Nemec et al. 1998; Stump et al. 1999). A series of studies showed an increased incidence of tumors in the offspring of mice exposed to arsenic from gestational day 8 through day 18(Waalkes et al. 2003, 2004a, 2004b, 2004c, 2006a, 2006b) (see Section 3.2.2.6 for further details). In humans, acute prenatal exposure to high doses of inorganic arsenic can result in miscarriage and early neonatal death (Bolliger et al. 1992; Lugo et al. 1969). Although several studies have reported marginal associations between prolonged low-dose human arsenic exposure and adverse reproductive outcomes, including spontaneous abortion, stillbirth, developmental impairment, and congenital malformation (Ahmad et al. 2001; Aschengrau et al. 1989; Chakraborti et al. 2003c; Hopenhayn-Rich et al. 2000; Nordström et al. 1978a, 1979b; Yang et al. 2003; Zierler et al. 1988), none of these studies have provided convincing evidence for such effects or information concerning possible dose-response relationships.

There is no evidence for differences in absorption of arsenic in children and adults. Ingestion of arsenic in dirt may be an important route of exposure for young children. A study that used a synthetic gastric juice designed to mimic gastric conditions in a 2-year-old child found that absorption of arsenic from contaminated soil was likely to be up to 5 times lower than the total concentration of arsenic in the soil (Williams et al. 1998). As previously mentioned, arsenic crosses the placenta and preferentially accumulates in the embryonic neuroepithelium. In addition, arsenic is known to be present in breast milk at low concentrations. Arsenic concentrations were low in human milk sampled from 88 mothers in the Faroe Islands (0.0001–0.0044 ppm), where the diet is predominantly seafood (exposures were primarily to “fish arsenic” [Grandjean et al. 1995]), in a population of Andean women (0.0008–0.008 ppm) exposed

to high concentrations of inorganic arsenic in drinking water (Concha et al. 1998b), and in a World Health Organization survey (0.00013–0.00082 ppm) (Somogyi and Beck 1993). There is no information in the literature describing storage of arsenic in maternal tissues. There is some evidence that metabolism of arsenic in children is less efficient than in adults. Children in two villages in Argentina ingesting large amounts of arsenic in their drinking water (200 μg/L) excreted about 49% inorganic arsenic and 47% DMA, compared to 32% inorganic arsenic and 66% DMA for the women in the study (Concha et al. 1998b). No PBPK models specifically targeted at fetuses, infants, or children, or pregnant or lactating women were found in the literature. There are no biomarkers that have been specifically identified for children exposed to arsenic. In addition, no unique interactions of arsenic with other chemicals have been identified in children.

The mechanism of toxic action of arsenic in the mammalian cell may involve inhibition of proliferation of cells (Dong and Luo 1993; Jha et al. 1992; Petres et al. 1977). In addition, high-dose arsenic impairs assembly and disassembly of microtubules, thus interfering with mitotic spindle formation and embryonal cell division (Léonard and Lauwerys 1980; Li and Chou 1992; Mottet and Ferm 1983). Arsenic compounds also cause chromosomal aberrations (Jha et al. 1992; Léonard and Lauwerys 1980), which may disrupt cell cycling. The direct toxic effects of high levels of arsenic in the developing embryo result not from a difference in the mechanism of toxicity during development, but rather from the existence of a unique target tissue, the neuroepithelium. The process of neurulation involves cell shape changes, cytokinesis, and cell adhesion, which are dependent upon cytoskeletal elements that are functionally affected by arsenic (Dallaire and Béliveau 1992; Edelman 1992; Gunn et al. 1992; Li and Chou 1992; Moriss-Kay et al. 1994; Schoenwolf and Smith 1990; Taubeneck et al. 1994). However, since arsenic is known to affect vasculature, and since altered placental and/or embryonal vasculature has been suggested as a mechanism leading to neural tube defects, the embryo may be sensitive to this manifestation of arsenic toxicity.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic

substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to arsenic are discussed in Section 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by arsenic are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10, Populations That Are Unusually Susceptible.

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Arsenic

Arsenic levels in blood, urine, hair, and nails have all been investigated and used as biological indicators of exposure to arsenic. Since arsenic is cleared from blood within a few hours (Tam et al. 1979b; Vahter 1983), measurements of blood arsenic reflect exposures only within the very recent past. Typical values in nonexposed individuals are <1 μg/L (Heydorn 1970; Hindmarsh and McCurdy 1986; Valentine et al.

1979). Consumption of medicines containing arsenic is associated with blood values of 100–250 μg/L, while blood levels in acutely toxic and fatal cases may be 1,000 μg/L or higher (Driesback 1980).

However, blood levels do not appear to be reliable indicators of chronic exposure to low levels of arsenic. For example, there was no correlation between the level of arsenic in blood of residents and the level of arsenic in drinking water in several U.S. communities where water levels ranged from about 6 to 125 μg/L (Valentine et al. 1979, 1981). Consequently, measurement of blood arsenic is not generally considered to be a reliable means of monitoring human populations for arsenic exposure.

As discussed in Section 3.4.4, most arsenic that is absorbed from the lungs or the gastrointestinal tract is excreted in the urine, mainly within 1–2 days. For this reason, measurement of urinary arsenic levels is generally accepted as the most reliable indicator of recent arsenic exposure, and this approach has proved useful in identifying above-average exposures in populations living near industrial point sources of arsenic (e.g., Milham and Strong 1974; Polissar et al. 1990). By the inhalation route, several researchers have found that there is a good quantitative correlation between the concentration of arsenic in workplace air (C_{air}, μg/m^3) and the concentration in the urine (C_{urine}, μg/L) of exposed workers. For example, Pinto et al. (1976) found a linear relationship for exposures ranging up to 150 μg/m^3, given by the following equation:

$$C_{air}=0.3\ C_{urine}$$

Enterline et al. (1987a) reinvestigated this relationship over a wider range of exposures (up to 3,500 μg/m^3), and found that the curve tended to be concave upward, as given by the following equation:

$$C_{air}=0.0064\ (C_{urine})^{1.94}$$

This indicates that at higher exposure levels, a higher fraction of the dose is excreted in urine, although the toxicokinetic basis for this is not certain. Numerous studies have used above-average urinary levels (i.e., higher than about 100 μg/L) as evidence of recent arsenic ingestion (e.g., Borgoño et al. 1980; Fincher and Koerker 1987; Franzblau and Lilis 1989; Goldsmith and From 1986; Kyle and Pease 1965; Valentine et al. 1981). Calderon et al. (1999) found a quantitative correlation between the log of the mean total urinary arsenic concentration/creatinine (TAs/c, μg/mg) of people living in areas with arsenic-contaminated drinking water sources and the log of the inorganic arsenic concentration in the drinking water (InAs, μg/L). The equation for the regression line is:

$$TAs/c=10^{-2.57} \times (InAs)^{0.63}$$

where -2.57 and 0.63 are the intercept and slope, respectively, for the regression of the log10-transformed data. Mixed model regression analysis showed that the log of estimated arsenic intake from drinking water (μg/day) is also a good predictor of TAs/c excretion (Calderon et al. 1999).

There is some indication that speciation of urinary arsenic may indicate the extent of past cumulative exposure to arsenic. Hsueh et al. (1998a) reported higher levels of DMA and MMA in the urine of individuals with higher cumulative past exposure to inorganic arsenic. Speciated urinary arsenic is also a recommended biomarker for recent inorganic arsenic exposure. Walker and Griffin (1998) used the EPA Exposure Assessment Model and a number of site-specific data covering environmental and biological factors to predict total and speciated urinary arsenic concentrations for children living near high levels of arsenic-contaminated soil. There was reasonable agreement between the measured and predicted speciated urinary arsenic concentrations.

An important limitation to the use of total urinary arsenic as a biomarker of exposure is that arsenobetaine is excreted (unmetabolized) in urine after ingestion of certain seafoods (Brown et al. 1990; Kalman 1987; Tam et al. 1982). Since "fish arsenic" is essentially nontoxic, analytical methods based on total urinary arsenic content may overestimate exposures to arsenic species that are of health concern. As discussed in Section 7.1, there are adequate methods for distinguishing arsenobetaine from other forms of arsenic in urine (inorganic, MMA, DMA), although these are not convenient to use as a routine screening method.

Arsenic tends to accumulate in hair and nails, and measurement of arsenic levels in these tissues may be a useful indicator of past exposures. Normal levels in hair and nails are 1 ppm or less (Choucair and Ajax 1988; Franzblau and Lilis 1989). These values may increase from several-fold to over 100-fold following arsenic exposure (Agahian et al. 1990; Bencko 2005; Bencko et al. 1986; de Peyster and Silvers 1995; EPA 1977a, 1981b; Karagas et al. 1996; Milham and Strong 1974; Valentine et al. 1979; Yamauchi et al. 1989) and remain elevated for 6–12 months (Choucair and Ajax 1988). Minimum exposure levels that produce measurable increases in arsenic levels in hair and nails have not been precisely defined. For hair, ingestion of 50–120 ppb of arsenic in drinking water produced only a marginal effect, but a clear increase was noted at 393 ppb (Valentine et al. 1979). A study of children living in a region polluted with arsenic derived from a power plant burning coal with a high arsenic content found a significant correlation between arsenic levels in hair and distance from the source of emission (Bencko and Symon 1977).

Inhalation exposure of workers to about 0.6 μg/m^3 of arsenic in air significantly increased average levels in nails (Agahian et al. 1990), although there was wide variation between individuals.

Analysis of hair may yield misleading results due to the presence of arsenic adsorbed to the external surface, but this can be minimized by collecting samples from close to the scalp or from unexposed areas and by washing the hair before analysis (e.g., Paschal et al. 1989). Similarly, extensive washing of nails is required to remove exogenous contamination (Agahian et al. 1990). The relationship between consumption of food items and levels of arsenic in toenails has been evaluated by MacIntosh et al. (1997) using standard multivariate regression models. This approach does not appear to be highly reliable, but may be sufficient for exploring associations between diet and disease. Kurttio et al. (1998) used linear regression models to show that there is a good association between arsenic concentration in hair (mg/kg) and total arsenic concentration in urine (μg/L), arsenic concentration in drinking water (μg/L) or daily intake of arsenic (μg/day). A 10 μg/L increase in the drinking water concentration or a 10–20 μg/day increase in daily arsenic intake corresponded to a 0.1 mg/kg increase in the arsenic concentration in hair. It is also important to note that the measurement of arsenic in hair and fingernails is a process not readily accessible to many clinical offices.

3.8.2 Biomarkers Used to Characterize Effects Caused by Arsenic

As discussed in Section 3.2, the characteristic pattern of skin changes caused by arsenic (hyperkeratinization, hyperpigmentation) is probably the most sensitive and diagnostic clinical indicator of chronic exposure to arsenic. However, no means has been developed for detecting these effects except by routine dermatological examination.

Peripheral neuropathy is another characteristic effect of arsenic exposure, and several researchers have investigated decreased nerve conduction velocity or amplitude as a biomarker for peripheral neuropathy. While effects can usually be detected in individuals with clinical signs of neuropathy (e.g., Goebel et al. 1990; Jenkins 1966; Le Quesne and McLeod 1977; Morton and Caron 1989; Murphy et al. 1981), effects are only marginal (EPA 1977a; Hindmarsh et al. 1977; Valentine et al. 1981) or undetectable (EPA 1981b; Kreiss et al. 1983) in exposed populations without obvious clinical signs of toxicity. This indicates that this approach is probably not sufficiently sensitive to detect neurological effects earlier than by standard neurological examination (Hindmarsh and McCurdy 1986). Also, decreases in nerve conduction velocity or amplitude are not specific for arsenic-induced neuropathy.

Arsenic is known to affect the activity of a number of enzymes, and some of these may have potential as biomarkers of effect. Most promising is the spectrum of effects caused by arsenic on the group of enzymes responsible for heme synthesis and degradation, including inhibition of coproporphyrinogen oxidase and heme synthetase (Woods and Fowler 1978; Woods and Southern 1989) and activation of heme oxygenase (Sardana et al. 1981). Menzel et al. (1998) has examined the *in vitro* induction of human lymphocyte heme oxygenase 1(HO1) as a biomarker of arsenite exposure. Arsenite did induce *de novo* synthesis of HO1 in human lymphoblastoid cells, but it has not been determined if the same response is induced *in vivo*. It has been shown in animals that these arsenic-induced enzymic changes result in increased urinary levels of uroporphyrin, coproporphyrin, and bilirubin (Albores et al. 1989; Woods and Fowler 1978), and it has been shown that these effects can be detected in the urine of arsenic-exposed humans (García-Vargas and Hernández-Zavala 1996). Therefore, altered urinary levels of these heme-related compounds could serve as a biomarker of effect. However, it is known that numerous other toxic metals also have similar effects on heme metabolism (Albores et al. 1989; Sardana et al. 1981; Woods and Southern 1989), so it is likely that these effects would not be specific for arsenic.

For more information on biomarkers for renal and hepatic effects of chemicals, see ATSDR/CDC Subcommittee Report on Biological Indicators of Organ Damage (Agency for Toxic Substances and Disease Registry 1990b) and for information on biomarkers for neurological effects, see OTA (1990).

3.9 INTERACTIONS WITH OTHER CHEMICALS

A number of researchers have found that arsenic compounds tend to reduce the effects of selenium (Hill 1975; Howell and Hill 1978; Kraus and Ganther 1989; Levander 1977; Miyazaki et al. 2003; Moxon et al. 1945; Schrauzer 1987; Schrauzer et al. 1978). Likewise, selenium can decrease the effects of arsenic, including clastogenicity (Beckman and Nordenson 1986; Biswas et al. 1999; Sweins 1983), delayed mutagenesis (Rossman and Uddin 2004), cocarcinogenesis (Uddin et al. 2005), cytotoxicity (Babich et al. 1989; Rössner et al. 1977; Styblo and Thomas 2001), and teratogenicity (Holmberg and Ferm 1969). The mechanism of this mutual inhibition of effects is not known, but may be related to the formation of a selenium-arsenic complex (seleno-bis [S-gluthionyl] arsinium ion; Gailer et al. 2002) that is excreted more rapidly than either arsenic or selenium alone (Cikrt et al. 1988; Hill 1975; Levander 1977; Levander and Baumann 1966) or due to selenium-induced changes in arsenic methylation (Styblo and Thomas 2001; Walton et al. 2003). There is little direct evidence that variations in selenium exposure in humans lead to significant increases or decreases in arsenic toxicity, although copper smelter workers who developed lung cancer had lower tissue levels of selenium than workers who did not develop lung tumors

(Gerhardsson et al. 1985, 1988). This suggests that selenium deficiency could significantly increase the risk of lung cancer following inhalation exposure to arsenic, but it is difficult to distinguish cause from effect in such a study. However, there is evidence that administration of selene can facilitate recovery from arsenic poisoning. In residents living in an area of Inner Mongolia with high levels of arsenic in drinking water, administration of 100–200 μg selenium/day in the form of selenium yeast and exposure to arsenic-free water for 14 months resulted in a greater improvement in clinical signs and symptoms, liver function, and EKG readings as compared to residents administered arsenic-free water only (Wuyi et al. 2001; Yang et al. 2002). An improvement in skin lesions was observed in 67 and 21% of the subjects in the selenium-supplemented and control groups (Yang et al. 2002). Additionally, the levels of arsenic in blood, hair, and urine were significantly lower after the 14-month period only in the selenium supplemented group.

The interaction between cigarette smoking, inhalation of arsenic, and the risk of lung cancer has not been extensively investigated. Smoking appeared to increase lung cancer risk synergistically (multiplicatively) in one study of smelter workers (Pershagen et al. 1981), although the data are not adequate to exclude a simple additive interaction (Thomas and Whittemore 1988). Cigarette smoking has been shown to increase the occurrence of lung cancer in people with high levels of arsenic in the drinking water (Chiou et al. 1995; Tsuda et al. 1995a). Suggestive evidence of a positive interaction between arsenic and benzo(a)pyrene has also been noted for induction of lung adenocarcinomas in hamsters (Pershagen et al. 1984).

Co-exposure to ethanol and arsenic may exacerbate the toxic effects of arsenic. Simultaneous exposure of rats to ethanol (10% in drinking water) and arsenic (dose not stated) for 6 weeks produced a significant increase in the concentration of arsenic in the kidney, a nonsignificant increase of arsenic in the liver and a significant increase in the concentration of glutathione in the liver, compared to rats treated with either ethanol or arsenic alone (Flora et al. 1997a, 1997b). Histological damage to the liver, but not the kidneys, was increased in rats treated with both ethanol and arsenic compared to those receiving only arsenic.

Studies of rats exposed to arsenic, lead, and cadmium, alone or in combination, have revealed mainly additive or subadditive effects on body weight, hematological parameters, and enzymes of heme synthesis (Mahaffey and Fowler 1977; Mahaffey et al. 1981). Similarly, studies of the tissue levels of arsenic in rats fed arsenic with or without lead or cadmium revealed only limited evidence of any toxicokinetic interactions (Mahaffey et al. 1981). Pretreatment of rats with a nontoxic dose of cadmium had no effect on the lethality of a high dose of arsenic and did not reduce arsenic-induced hepatotoxicity (Hochadel and

Waalkes 1997). These data do not suggest that arsenic toxicity is likely to be significantly influenced by concomitant exposure to these metals. However, supplementation with zinc or chromium may be useful in reducing chronic arsenism. Arsenic has been shown to cause an increase in total plasma cholesterol; co-administration of chromium(III) counteracts this effect (Aguilar et al. 1997). Pretreatment of mice with zinc, at least 24 hours before injection with arsenic-73, reduced arsenic retention compared to controls that did not receive the zinc pretreatment or received it only a short time before the administration of arsenic (Kreppel et al. 1994). Zinc is an inducer of metallothionein, but this induction does not appear to be the mechanism that reduces arsenic toxicity because other inducers of metallothionein did not reduce arsenic toxicity and arsenic elimination was increased by the zinc pretreatment.

Since methylation of arsenic is a detoxification mechanism, it is possible that chemicals that interfere with the methylation process could increase toxicity. This is supported by studies in animals in which reagents that inhibit methylation enzymes (e.g., periodate-oxidized adenosine) caused an increase in tissue levels of inorganic arsenic (Marafante and Vahter 1986; Marafante et al. 1985). Similarly, cellular glutathione levels appear to play a role in the methylation process, and treatment with reagents (e.g., phorone) that decrease glutathione levels increases arsenic toxicity (Buchet and Lauwerys 1987). Inadequate dietary intake of methionine, choline, or protein may also exacerbate arsenic toxicity. Rabbits pretreated with diets low in choline, methionine, or protein showed a significant increase in tissue retention of arsenic and a significant decrease in the excretion of dimethylarsinic acid (Vahter and Marafante 1987). The increased retention of arsenic in rabbits fed these deficient diets is likely to be due to a reduction in arsenic methylation. Thus, the toxic effects of chronic arsenic ingestion may be increased in populations that are also subject to malnutrition.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to arsenic than will most persons exposed to the same level of arsenic in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of arsenic, or compromised function of organs affected by arsenic. Populations who are at greater risk due to their unusually high exposure to arsenic are discussed in Section 6.7, Populations with Potentially High Exposures.

No studies were located that identified an unusual susceptibility of any human subpopulation to arsenic. Several studies have evaluated possible sex-related differences in arsenic toxicity and carcinogenesis (Aposhian et al. 2000a, 2000b; Calderon et al. 1999; Loffredo et al. 2003; Mandal et al. 2001; Watanabe et al. 2001), but have not consistently identified differences. However, since the degree of arsenic toxicity may be influenced by the rate and extent of its methylation in the liver (see Section 3.4.3), it seems likely that some members of the population might be especially susceptible because of lower than normal methylating capacity. Studies of exposed humans in Taiwan suggested that subjects with lower secondary methylation indices have an increased risk of bladder cancer (Chen et al. 2003) and peripheral vascular disease (Tseng et al. 2005), particularly in subjects with high exposure levels. Reduced hepatic methylation could result from dietary deficiency of methyl donors such as choline or methionine (Buchet and Lauwerys 1987; Vahter and Marafante 1987), although this is unlikely to be a concern for most people in the United States. There is evidence that methylation capacity can vary greatly among individuals (e.g., Buchet et al. 1981a; Foà et al. 1984; Hopenhayn-Rich et al. 1996b; Tam et al. 1979b), but the basis of this variation and its impact on human susceptibility have not been fully established. There is some evidence that low dietary protein intake and possibly other nutritional deficiencies can decrease arsenic methylation (Steinmaus et al. 2005a). Recently, Heck et al. (2007) examined whether the capacity to methylate arsenic differs by nutrient intake in a cohort of 1,016 Bangladeshi adults exposed to arsenic in drinking water. The results showed that higher intakes of cysteine, methionine, calcium, protein, and vitamin B-12 were associated with lower percentages of inorganic arsenic and higher ratios of MMA to inorganic arsenic in urine. In addition, higher intakes of niacin and choline were associated with higher DMA/MMA ratios, after adjustment for sex, age, smoking, total urinary arsenic, and total energy intake. The issue of increased susceptibility to arsenic due to poor nutrition was discussed by NRC (2001), it was concluded that, with regard to skin effects, studies of cohorts from India, Bangladesh, and Taiwan suggest that nutrition plays an important role in arsenic toxicity. On the other hand, studies in other regions of the world (i.e., Chile) involving populations with much better nutrition argue against poor nutrition having a major impact on arsenic toxicity.

Various genetic polymorphisms also seem to play a role in arsenic-induced toxicity. For example, a study of 85 lung cancer patients and 108 healthy controls in northern Chile reported that there was a nonstatistically significant difference for the frequency of the GSTM1 null genotype between the healthy and lung cancer patients stratified by gender and smoking status. The same results were observed for the MspI CYP450 1A1 polymorphism (Adonis et al. 2005). Hsueh et al. (2005) examined the association of four polymorphisms: NAD(P)H oxidase, manganese superoxide dismutase (MnSOD), catalase, and endothelial nitric oxide synthase (eNOS) with arsenic related hypertension risk among 79 hypertensive

cases and 213 controls in an arseniasis-hyperendemic area in Taiwan. The results showed that MnSOD polymorphism significantly increased the risk of hypertension regardless of exposure to arsenic. NAD(P)H oxidase and eNOS polymorphisms were significantly associated with increased risk of hypertension in subjects with higher cumulative arsenic exposure (≥10.5 mg/L x year), whereas catalase polymorphism was not associated with hypertension. The results also showed that the association between MnSOD, NAD(P)H oxidase, and eNOS polymorphisms and risk of hypertension were more pronounced in subjects with high triglyceride level. A study of a population of West Bengal, India, exposed to arsenic via drinking water reported that the frequencies of null genotype in GSTT1 were 13.52 and 12.92% in skin-symptomatic and skin-asymptomatic individuals, and GSTM1 null genotype were 13.90 and 22.47% in skin-symptomatic and skin-asymptomatic individuals, respectively (Ghosh et al. 2006). Compared to those with GSTM1 null genotype, subjects with GST1-positive (at least one allele) had significantly higher risk of arsenic-induced skin lesions. Recently, Steinmaus et al. (2007) investigated urinary arsenic methylation patterns and genetic polymorphisms in methylenetetrahydro-folate reductase (MTHFR) and GST in 170 subjects (139 males) from an arsenic-exposed region in Argentina. MTHFR is a key enzyme in the metabolism of folate and has been linked to arsenic metabolism and toxicity (NRC 1999). Steinmaus et al. (2007) found that subjects with the TT/AA variant of MTHFR 677/1298 (associated with lower MTHFR activity) excreted a significantly higher proportion on ingested arsenic as inorganic arsenic and a smaller proportion as DMA(V). The study also reported that women with null genotype of GSTM1 excreted a significantly higher proportion of arsenic as monomethylarsenate than women with the active genotype. The study also found no association between polymorphisms in GSTT1 and arsenic methylation.

There is a report that described severe arsenic-induced neuropathy that developed only in a 5,10-methylenetetrahydrofolate-reductase (MTHFR) deficient member of a family that had been exposed to arsenic (Brouwer et al. 1992). The authors suggest that the MTHFR deficiency in this girl might explain the fact that of all the family members exposed to arsenic, only she developed severe clinical signs of arsenic poisoning. Liver disease does not appear to decrease methylation capacity in humans, at least at low levels of arsenic exposure (Buchet et al. 1982; Geubel et al. 1988).

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to arsenic. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to arsenic. When specific

exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide specific information about treatment following exposures to arsenic:

Tintinalli JE, Ruiz E, Krone RL, eds. 1996. Emergency medicine. A comprehensive study. American College of Emergency Physicians. 4th ed. New York, NY: The McGraw-Hill Companies, Inc.

Goldfrank RL, Flomenbaum NE, Lewin NA, et al., eds. 1998. Goldfrank's toxicologic emergencies. 6th ed. Stamford, CT: Appleton and Lange.

Ellenhorn MJ. 1997. Ellenhorn's medical toxicology. Diagnosis and treatment of human poisoning. Baltimore, MD: Williams & Wilkins.

3.11.1 Reducing Peak Absorption Following Exposure

No data were located regarding the reduction of absorption after inhalation exposure to arsenic.

There are a number of methods for reducing absorption of arsenic following oral exposure. In cases of acute high-dose exposure, the removal of arsenic from the gastrointestinal tract may be facilitated by gastric lavage, stomach intubation, induced emesis, or use of cathartics (saline, sorbitol) within a few hours after ingestion (Agency for Toxic Substances and Disease Registry 1990a; Aposhian and Aposhian 1989; Campbell and Alvarez 1989; Driesback 1980; Ellenhorn and Barceloux 1988; EPA 1989e; Haddad and Winchester 1990; Kamijo et al. 1998; Stutz and Janusz 1988). However, the efficacy of several of these methods has been questioned by some authors, and in some cases, the treatments may be contraindicated. For example, vomiting and diarrhea often occur soon after ingesting arsenic, and therefore, use of an emetic or cathartic may not be necessary. Also, emesis should not be induced in obtunded, comatose, or convulsing patients (Campbell and Alvarez 1989; Ellenhorn and Barceloux 1988; EPA 1989e), and saline cathartics should be used with caution in patients with impaired renal function (Campbell and Alvarez 1989). Vantroyen et al. (2004) described a case of a massive arsenic trioxide overdose that was successfully treated by continuous gastric irrigation with sodium bicarbonate, forced diuresis, and administration of BAL and DMSA. Treatments of this sort are unlikely to be required following low-level exposures.

Another possible approach for reducing absorption following oral exposure is to administer substances that bind the arsenic in the gastrointestinal tract. For example, activated charcoal is sometimes used for this purpose (Campbell and Alvarez 1989; EPA 1989e; Stutz and Janusz 1988), although the effectiveness of this treatment is not well established. Because pentavalent arsenic is a phosphate analogue,

administration of phosphate-binding substance such as aluminum hydroxide might possibly be useful, but this has not been investigated. Sulfhydryl compounds might be given to bind trivalent arsenic, but it seems unlikely that these would be effective under the acid conditions in the stomach, and it is not clear that such complexes would have reduced gastrointestinal absorption.

Following dermal or ocular exposure to arsenic, several measures can be taken to minimize absorption. All contaminated clothing should be removed, and contacted skin should be immediately washed with soap and water. Eyes that have come in contact with arsenic should be flushed with copious amounts of clean water (EPA 1989e; Stutz and Janusz 1988).

3.11.2 Reducing Body Burden

Acute arsenic intoxication may require treatment with chelating agents such as dimercaprol (BAL) and D-penicillamine. Although body burden is not necessarily reduced, these chelators bind free arsenic and serve to reduce the body's pool of biologically active arsenic. Chelation therapy is most effective when instituted within a few hours after exposure, and efficacy decreases as time after exposure increases (Agency for Toxic Substances and Disease Registry 1990a; Kamijo et al. 1998; McFall et al. 1998; Peterson and Rumack 1977).

In general, chelating agents should be used with caution, since they may have serious side effects such as pain, fever, hypotension, and nephrotoxicity (Ellenhorn and Barceloux 1988). Some water-soluble and less toxic analogues of BAL such as dimercaptosuccinic acid (DMSA), dimercaptopropyl phthalamadic acid (DMPA), and dimercaptopropane sulfonic acid (DMPS) are currently under investigation and may prove to be promising treatments for arsenic poisoning (Agency for Toxic Substances and Disease Registry 1990a; Aposhian and Aposhian 1989; Aposhian et al. 1997; Guha Mazumder 1996; Kreppel et al. 1995). However, a randomized placebo trial of 2,3-dimercaptosuccinic acid as a therapy for chronic arsenosis due to drinking contaminated water found no significant difference between patients treated with 2,3-dimercaptosuccinic acid and those treated with a placebo (Guha Mazumder et al. 1998a). N-acetylcysteine has been used in animals to chelate arsenic (Haddad and Winchester 1990), and a human case study reported N-acetylcysteine to be successful in treating a case of arsenic poisoning that was not responding well to BAL treatment (Martin et al. 1990). Vantroyen et al. (2004) described a case of a massive arsenic trioxide overdose that was successfully treated by continuous gastric irrigation with sodium bicarbonate, forced diuresis, and administration of BAL and DMSA.

As discussed in Section 3.4.3, once arsenic has been absorbed into the blood stream, it undergoes methylation to yield MMA and DMA. These forms of arsenic are less toxic than inorganic arsenic and are cleared from the body by excretion in the urine. Therefore, if it were possible to enhance arsenic methylation, both body burden and toxicity of arsenic might be reduced. However, experimental evidence in animals and humans suggests that arsenic methylation is not enhanced to any significant degree by supplementation with methylation cofactors (Buchet and Lauwerys 1987; Buchet et al. 1982), presumably because it is enzyme level and not cofactor availability that is rate limiting in arsenic methylation.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

It is generally thought that trivalent arsenic exerts its toxic effects mainly by complexing with sulfhydryl groups in key enzymes within the body, thereby inhibiting critical functions such as gluconeogenesis and DNA repair (Aposhian and Aposhian 1989; Li and Rossman 1989). Therefore, administration of sulfhydryl-containing compounds soon after exposure could provide alternative target molecules for arsenic, and prevent inhibition of enzyme functions. In fact, many of the chelating agents discussed above (BAL, DMSA, DMPA, DMPS, N-acetylcysteine) contain sulfhydryl groups, and this may account for their efficacy.

The mechanism by which pentavalent arsenic acts is less certain. Since pentavalent arsenic is reduced in the body to the trivalent state, pentavalent arsenic may act in a similar manner as described above for trivalent arsenic. If this is the case, efforts to inhibit the reduction of pentavalent arsenic would decrease its toxicity. However, no methods are currently recognized for blocking this reduction. Pentavalent arsenic may also exert effects by acting as a phosphate analogue. As a phosphate analogue, pentavalent arsenic could potentially affect a number of biological processes, including ATP production, bone formation, and DNA synthesis. However, any effort to interfere in normal phosphate metabolism could produce serious side effects, and no method is known for selectively interfering with arsenate metabolism.

3.12 ADEQUACY OF THE DATABASE

Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of arsenic is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the

initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of arsenic.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

3.12.1 Existing Information on Health Effects of Arsenic

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to inorganic and organic arsenic are summarized in Figures 3-13 and 3-14, respectively. The purpose of this figure is to illustrate the existing information concerning the health effects of arsenic. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need". A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

As shown in Figure 3-13, there is a substantial database on the toxicity of inorganic arsenicals, both in humans and in animals. The oral route has been most thoroughly investigated, and reports are available on most end points of concern following acute, intermediate, and chronic exposure. The inhalation route has also been studied extensively, mainly in humans, with special emphasis on lung cancer. A number of noncancer end points have also been studied following inhalation exposure, but information on these effects is less extensive. Limited information on the effects of dermal exposure is also available in both humans and animals, focusing mainly on direct irritancy and dermal sensitization reactions. The absence of studies on other effects of inorganic arsenic following dermal exposure is probably not a critical data need, since dermal uptake of inorganic arsenic appears to be sufficiently limited that other routes of exposure (oral or inhalation) would almost always be expected to be of greater concern.

Figure 3-13. Existing Information on Health Effects of Inorganic Arsenic

	Death	Acute	Intermediate	Chronic	Systemic: Immunologic/Lymphoretic	Systemic: Neurologic	Systemic: Reproductive	Systemic: Developmental	Systemic: Genotoxic	Cancer
Inhalation	●	●	●	●	●	●		●	●	●
Oral	●	●	●	●	●	●	●	●	●	●
Dermal		●	●	●	●	●				

Human

	Death	Acute	Intermediate	Chronic	Systemic: Immunologic/Lymphoretic	Systemic: Neurologic	Systemic: Reproductive	Systemic: Developmental	Systemic: Genotoxic	Cancer
Inhalation	●	●	●		●	●		●	●	●
Oral	●	●	●	●	●	●	●	●	●	●
Dermal	●	●	●		●					●

Animal

● Existing Studies

Figure 3-14. Existing Information on Health Effects of Organic Arsenic

	Death	Acute	Intermediate	Chronic	Immunologic/Lymphoretic	Neurologic	Reproductive	Developmental	Genotoxic	Cancer
	Systemic									
Inhalation		●		●		●				
Oral		●				●				
Dermal						●				

Human

	Death	Acute	Intermediate	Chronic	Immunologic/Lymphoretic	Neurologic	Reproductive	Developmental	Genotoxic	Cancer
	Systemic									
Inhalation	●	●								
Oral	●	●	●	●	●	●	●	●	●	●
Dermal		●								

Animal

● Existing Studies

As shown in Figure 3-14, very little information is available on the effects of organic arsenic compounds in humans, although there are a number of studies in animals. These studies mainly involve the oral route, since all of these compounds are nonvolatile solids, although a few acute inhalation studies have been performed. Limited information is available on acute dermal lethality and dermal irritancy of some organic arsenicals, but data are lacking on other effects of organic arsenicals following dermal exposure. As discussed previously, in evaluating the adequacy of the database on arsenic, it is important to keep in mind that most studies in animals indicate that they are quantitatively less sensitive to arsenic than humans. For this reason, data from animal studies should be used to draw inferences about effects in humans only with caution.

3.12.2 Identification of Data Needs

Acute-Duration Exposure.

Inorganic Arsenicals. There is only limited information on the effects of acute inhalation exposure to arsenic in humans, but the chief symptoms appear to be irritation of the respiratory and gastrointestinal tracts (Beckett et al. 1986; Bolla-Wilson and Bleecker 1987; Dunlap 1921; Ide and Bullough 1988; Morton and Caron 1989; Pinto and McGill 1953). Quantitative data are lacking, but effects generally appear to be mild even at high-exposure levels. On this basis, it seems that risks of acute effects are probably low for inhalation exposures in the environment or near waste sites. Research to obtain a quantitative acute inhalation NOAEL value that could be used to derive an acute inhalation MRL would, therefore, be useful but not critical. There are numerous case studies in humans on the acute oral toxicity of arsenic, and the main end points (gastrointestinal irritation, pancytopenia, hepatic injury, neuropathy) are well characterized (Armstrong et al. 1984; Fincher and Koerker 1987). An acute oral MRL of 0.005 mg As/kg/day was derived for inorganic arsenic based on a LOAEL for gastrointestinal symptoms and facial edema reported by Mizuta et al. (1956). Additional studies to define an acute oral NOAEL would be useful to reduce uncertainty in the MRL derivation. Acute dermal exposure is unlikely to cause serious systemic injury, but it can lead to contact dermatitis and skin sensitization (Holmqvist 1951; Pinto and McGill 1953). However, available data do not permit a quantitative estimate of the concentration of arsenic on the skin or in air, dust, soil, or water that causes these effects. Further research would be valuable to obtain a quantitative NOAEL for direct dermal effects, since humans may have dermal contact with contaminated soil or water near hazardous waste sites.

Organic Arsenicals. Information on the acute toxicity of organic arsenicals in humans is limited to reports of gastrointestinal irritation in individuals ingesting pesticides containing organic arsenicals (Lee et al. 1995; Shum et al. 1995); these case reports provide limited dosing information. Acute lethality and systemic toxicity data exist for several compounds by inhalation, oral, and dermal exposure of animals. Inhalation data are limited to a lethality study of rats and mice exposed to MMA or DMA that reported respiratory and ocular irritation (Stevens et al. 1979). The oral acute studies consist of lethality studies for MMA (Gur and Nyska 1990; Jaghabir et al. 1988; Kaise et al. 1989), DMA (Kaise et al. 1989), and roxarsone (Kerr et al. 1963; NTP 1989b), systemic toxicity studies (or longer-term studies reporting effects within the first 2 weeks of exposure) for MMA (Irvine et al. 2006), DMA (Ahmad et al. 1999a; Chernoff et al. 1990; Cohen et al. 2001; Crown et al. 1987; Irvine et al. 2006; Kavlock et al. 1985; Rogers et al. 1981; Zomber et al. 1989), or roxarsone (NTP 1989b). For MMA, the available data suggest that the gastrointestinal tract may be the most sensitive target of toxicity; however, the study identifying the lowest LOAEL (Irvine et al. 2006) involved bolus administration and this is not an appropriate exposure route to estimate human risk for gastrointestinal effects following environmental exposure to MMA. The available animal studies for DMA have examined urinary bladder (Cohen et al. 2001) and developmental toxicity (Chernoff et al. 1990, Irvine et al. 2006; Kavlock et al. 1985; Rogers et al. 1981). For DMA, acute-duration studies in rats suggest that the urinary bladder is the most sensitive target of toxicity in rats (Cohen et al. 2001); however, there is evidence from longer-term studies that rats may be more sensitive than humans and other species for bladder effects. Thus, rat data were not considered as the basis of an acute-duration oral MRL for DMA. Other effects observed following acute exposure to DMA include developmental and maternal effects in mice (Kavlock et al. 1985; Rogers et al. 1981) and rabbits (Irvine et al. 2006) and diarrhea and vomiting in dogs receiving a bolus dose of DMA (Zomber et al. 1989). An acute-duration oral MRL was not derived for DMA because it is not known if systemic effects would occur at lower doses than the developmental effects. For roxarsone, the available data suggest that the most sensitive effect following acute oral exposure is neuropathy observed in pigs (Kennedy et al. 1986; Rice et al. 1985). At the only dose tested in this study, tremors, clonic convulsions, and equivocal evidence of myelin degeneration were observed; these were considered serious effects and not suitable for the derivation of an acute-duration oral MRL for roxarsone. Additional studies are needed for MMA, DMA, and roxarsone that examine a variety of end points in several species; studies for roxarsone should also include examination of neurological end points, which would be useful for identifying the critical targets of toxicity and establishing dose-response relationships.

Intermediate-Duration Exposure.

Inorganic Arsenicals. Intermediate-duration inhalation exposure of humans to arsenic appears to result in respiratory tract irritation (occasionally including perforation of the nasal septum) and mild gastrointestinal tract irritation (Ide and Bullough 1988). Quantitative data are too limited (only one study, of one individual) to derive an intermediate-duration inhalation MRL. Further studies to define the NOAEL for intermediate-duration inhalation exposure of humans would be valuable, since humans could be exposed to arsenic-containing airborne dusts near smelters, chemical plants, or waste sites. Effects of intermediate-duration oral exposure are similar to those of acute oral exposure, but may also include development of vascular injury and a characteristic group of skin changes (Franzblau and Lilis 1989; Holland 1904; Wagner et al. 1979). Most studies indicate that these effects occur at doses of about 0.05 mg As/kg/day or higher, but the data do not provide a firm basis for identifying the intermediate-duration NOAEL. For this reason, no intermediate-duration oral MRL has been derived. Further studies to establish the NOAEL would be valuable, since humans could have intermediate-duration oral exposures to arsenic through ingestion of contaminated soil or water near smelters, chemical factories, or waste sites. Since dermal effects appear to be restricted to acute irritancy, intermediate-duration dermal studies are probably not essential.

Organic Arsenicals. No information was located on the intermediate-duration toxicity of organic arsenicals in humans. Several studies have examined the intermediate-duration oral toxicity of MMA; dietary exposure studies in rats and mice (Arnold et al. 2003) identify the gastrointestinal tract as the most sensitive target. Diarrhea and lesions in the cecum, colon, and rectum have been observed. The rat 13-week study (Arnold et al. 2003) was used as the basis of the MRL. Because rats appear to be more sensitive to the toxicity of DMA, rat studies were not considered for MRL derivation. The only non-rat study was a chronic-duration dog study reporting effects during the first 51 weeks of exposure (Zomber et al. 1989); these effects included diarrhea and vomiting. However, because DMA was administered via capsule, this study was not considered adequate for derivation of an MRL. Additional studies are needed for DMA to identify critical targets of toxicity and establish dose-response relationships in non-rat species. The available data for roxarsone suggest that neurotoxicity in pigs is the most sensitive end point. One of the two available neurotoxicity studies in pigs (Edmonds and Baker 1986) did not include sensitive tests of toxicity and was not considered for MRL derivation; the other study identified a serious LOAEL at the only dose tested and thus, was not suitable for MRL derivation. Several comprehensive studies examined the toxicity of roxarsone in rats and mice (NTP 1989b). Renal tubular damage in rats was the most sensitive end point (NTP 1989b); however, the LOAEL for this effect was 9 times higher

than the dose associated with neurotoxicity in pigs. Additional studies are needed to establish a no effect level for neurotoxicity in pigs, which could be used to derive an intermediate duration MRL for roxarsone. Further studies on the intermediate-duration inhalation and dermal toxicity of these compounds would be valuable, especially in humans, since people may be exposed to organic arsenicals during their manufacture or use, or from materials deposited in waste sites.

Chronic-Duration Exposure and Cancer.

Inorganic Arsenicals. The target tissues of chronic-duration exposure of humans to inorganic arsenic are the same as for intermediate-duration exposure for both the oral and inhalation routes. Effects of dermal exposure appear to be restricted to direct irritation of exposed surfaces. Therefore, chronic-duration studies are probably not essential for the dermal route. Quantitative data from one study identify an inhalation exposure level of about 0.1 mg As/m^3 as the LOAEL for skin changes (Perry et al. 1948), but because there are no additional supporting studies and a NOAEL is not clearly established, a chronic-duration inhalation MRL has not been derived. Additional studies in humans to define the chronic inhalation NOAEL for dermal or other effects would be valuable, since humans may be chronically exposed to arsenic dusts in air near smelters, chemical factories, or waste sites. Chronic oral exposure data from studies in humans indicate that the LOAEL for skin lesions and other effects is probably about 0.01–0.02 mg As/kg/day (10–20 μg As/kg/day), and that the NOAEL is probably between 0.0004 and 0.0009 mg As/kg/day (0.4–0.9 μg As/kg/day) (Cebrián et al. 1983; EPA 1981b; Hindmarsh et al. 1977; Tseng 1977; Tseng et al. 1968). The NOAEL of 0.0008 mg As/kg/day from the study by Tseng et al. (1968) is appropriate for derivation of a chronic-duration oral MRL, but an uncertainty factor of 3 was required to account for the fact that the population that constituted the no-effect group were relatively young (possibly decreasing the ability to detect dermal or other effects that increase in prevalence with age). Another issue that needs to be acknowledged, which is common to ecological studies and contributes to uncertainty, is the fact that individual doses were not available and were calculated from group mean arsenic concentrations in well water using estimated water intake parameters. For this reason, further epidemiological studies that do not rely on an ecological-based exposure assessment that would provide additional support for the threshold dose for arsenic in humans would be valuable.

There are numerous studies in humans that support the carcinogenic effects of inorganic arsenic from inhalation exposure (Enterline et al. 1987a, 1987b, 1995; Järup and Pershagen 1991; Järup et al. 1989; Lee-Feldstein 1986; Welch et al. 1982) and oral exposure (Chen et al. 1986, 1988b, 1992; Chiou et al. 1995; Ferreccio et al. 1996; Hsueh et al. 1995; Lander et al. 1975; Liu and Chen 1996; Lüchtrath 1983;

Smith et al. 1992; Tseng 1977; Tseng et al. 1968; Yu et al. 1992; Zaldívar 1974; Zaldívar et al. 1981). Quantitative slope factors have been derived for both routes. There is a noticeable absence, however, of 2-year animal carcinogenicity studies for either the inhalation or oral route of exposure (Chan and Huff 1997). In light of the ongoing controversy over the reasons for the absence of a carcinogenic effect in animals, it seems prudent to firmly establish a negative effect in a 2-year study. The carcinogenic effects of chronic dermal exposure to inorganic arsenicals have not been studied, but dermal exposure is a relatively minor route of exposure, and these studies would not be a top priority.

The mechanism of arsenic carcinogenicity is not known, although the current view is that it functions mainly as a promoter or cocarcinogen. Further studies on the mechanism of arsenic toxicity would be particularly valuable to improve our ability to evaluate human cancer risks from inhalation or oral exposures that might occur near waste sites. Also, mechanistic studies could help in the evaluation of cancer risks from organic derivatives (see below).

Organic Arsenicals. There is very little information on the chronic toxicity of organic arsenicals in humans. One study of workers exposed to arsanilic acid did not identify any adverse effects, but no systematic, clinical, or toxicological examinations of exposed people were performed (Watrous and McCaughey 1945). Chronic toxicity studies are available for rats, mice, and dogs exposed to MMA (Arnold et al. 2003; Waner and Nyska 1988), DMA (Arnold et al. 2006; Zomber et al. 1989), and roxarsone (NTP 1989b; Prier et al. 1963). Chronic exposure to MMA results in diarrhea in rats, mice, and dogs (Arnold et al. 2003; Waner and Nyska 1988) and an increase in progressive nephropathy in rats and mice (Arnold et al. 2003). The increased incidence of progressive nephropathy was used as the basis of the chronic-duration oral MRL for MMA. For DMA, chronic exposure also resulted in an increased incidence of diarrhea and vomiting in dogs (Zomber et al. 1989) and an increased incidence of vacuolization in the urinary bladder and progressive nephropathy in mice (Arnold et al. 2006). The vacuolization in the urinary bladder was used as the basis of a chronic-duration oral MRL for DMA. The available data for chronic-exposure to roxarsone were considered inadequate for derivation of an MRL. The highest doses tested in the rat, mouse, and dog studies (NTP 1989b; Prier et al. 1963) were NOAELs. Intermediate-duration studies identify neurotoxicity in pigs as the most sensitive end point; this has not been adequately examined following chronic exposure and studies are needed.

No information was located on carcinogenic effects of organic arsenicals in humans. The carcinogenic potential of MMA (Arnold et al. 2003), DMA (Arnold et al. 2006), and roxarsone (NTP 1989b) following oral exposure has been investigated in rats and mice. No evidence of carcinogenicity was observed

following oral exposure to MMA (Arnold et al. 2003) and equivocal evidence of carcinogenicity was found in male rats, with no evidence of carcinogenicity in female rats or in male or female mice orally exposed to roxarsone (NTP 1989b). Oral exposure to DMA resulted in an increased incidence of urinary bladder tumors in rats and no evidence of carcinogenicity in mice (Arnold et al. 2006). However, there is concern that the rat is not a good model to assess the carcinogenic potential of DMA in humans due to species differences in the toxicokinetic properties of DMA. No information was located on the carcinogenicity of organic arsenicals following inhalation or dermal exposure. Studies of humans exposed in the workplace would provide valuable information on the carcinogenic potential of organic arsenicals, particularly DMA. Studies on cancer risk following inhalation and dermal exposure to organic arsenicals are would be useful since these are possible routes of exposure for humans.

Genotoxicity.

Inorganic Arsenicals. There are several studies that suggest that inorganic arsenic may cause genotoxicity (mainly chromosomal effects) in exposed humans (Burgdorf et al. 1977; Nordenson et al. 1978), and this is supported by numerous studies in animals (Datta et al. 1986; DeKnudt et al. 1986; Nagymajtényi et al. 1985) and cultured cells (Beckman and Nordenson 1986; Casto et al. 1979; DiPaolo and Casto 1979; Lee et al. 1985; Nakamuro and Sayato 1981; Nishioka 1975; Oberly et al. 1982; Okui and Fujiwara 1986; Sweins 1983; Ulitzur and Barak 1988; Zanzoni and Jung 1980). The mechanism of genotoxicity is not known, but may be due to the ability of arsenite to interfere with DNA repair (Li and Rossman 1989) or to alter apoptosis (Pi et al. 2005) or the ability of arsenate to act as a phosphate analog. Further studies to improve our understanding of the mechanism of genotoxicity would be valuable, since this could aid in the understanding of arsenic-induced cancer risk.

Organic Arsenicals. For organic arsenicals, *in vitro* genotoxicity studies are available for arsenobetaine (Eguchi et al. 1997; Oya-Ohta et al. 1996), MMA (Chun and Killeen 1989a, 1989b, 1989c, 1989d; Eguchi et al. 1997; Oya-Ohta et al. 1996), DMA (Eguchi et al. 1997; Endo et al. 1992; Kato et al. 1994; Kawaguchi et al. 1996; Kitamura et al. 2002; Kuroda et al. 2004; Moore et al. 1997a; Oya-Ohta et al. 1996; Rasmussen and Menzel 1997; Rin et al. 1995; Tezuka et al. 1993; Ueda et al. 1997; Yamanaka et al. 1989b, 1993, 1995, 1997), and roxarsone (Matthews et al. 1993; NTP 1989b; Storer et al. 1996) and *in vivo* studies are available for DMA (Kashiwada et al. 1998; Yamanaka and Okada 1994; Yamanaka et al. 1989a, 1989b, 1993, 2001). The results of these studies suggest that DMA and roxarsone are clastogenic and can cause DNA strand breaks. Additional *in vivo* studies are needed to evaluate the genotoxic potential of MMA and roxarsone.

Reproductive Toxicity.

Inorganic Arsenicals. Several studies have examined reproductive function in populations living in Bangladesh or India exposed to high levels of arsenic in drinking water and found increases in spontaneous abortions/stillbirths or preterm births (Ahmad et al. 2001; von Ehrenstein et al. 2006); another study in U.S. women did not find an increase in adverse reproductive outcomes (Aschengrau et al. 1989). Available animal studies did not find evidence for reproductive effects following inhalation or oral exposure (Holson et al. 1999, 2000), except for a trend toward decreased pups per litter in mice in a 3-generation study (Schroeder and Mitchener 1971) that is consistent with embryolethality observed in developmental studies of inorganic arsenic. Studies on spermatogenesis and reproductive success in arsenic-exposed workers would be valuable in evaluating whether there are significant reproductive risks of arsenic in humans, and this could be further strengthened by studies including histopathological examination of reproductive tissues (which was not done in the existing studies) in animals.

Organic Arsenicals. No information was located on reproductive effects of organic arsenicals in humans and no inhalation or dermal exposure animal studies were located. Intermediate- and chronic-duration oral studies for MMA (Arnold et al. 2003), DMA (Arnold et al. 2006), and roxarsone (NTP 1989b) have not reported histological damage to reproductive tissues. Decreases in pregnancy rate and male fertility index were observed in a two-generation study in rats (Schroeder 1994) and a single generation study in mice (Prukop and Savage 1986) exposed to MMA; the poor reporting in the Prukop and Savage (1986) study limits its usefulness in assessing reproductive toxicity. However, in the two-generation study, the differences between control and exposed rats were not statistically different; the effect was considered biologically significant because effects observed in the exposed rats were outside the range found in historical controls. Another reproductive performance study to confirm these results would be useful. No reproductive effects were observed in a two generation study in rats exposed to DMA (Rubin et al. 1989).

Developmental Toxicity.

Inorganic Arsenicals. There are several epidemiological studies that suggest that inhalation (Ihrig et al. 1998; Nordström et al. 1978a, 1978b, 1979a, 1979b) or oral (Hopenhayn et al. 2003a; Yang et al. 2003) exposure to inorganic arsenic might increase the risk of low birth weight, congenital defects, or abortion in exposed women. These studies do not establish that arsenic was responsible, since all involved exposures to other chemicals or risk factors, but do suggest that additional studies on developmental

parameters in humans exposed to arsenic would be valuable in determining whether this is an effect of concern. Other human studies have not found significant associations between arsenic levels in drinking water and increased neonatal deaths or infant mortality (von Ehrenstein et al. 2006) or the increase in congenital heart defects (Zierler et al. 1988) or neural tube defects (Brender et al. 2006). Studies in animals support the view that oral, inhalation, and parenteral exposure to inorganic arsenic can all increase the incidence of fetotoxicity and teratogenicity, although this appears to occur only at doses that are toxic or even lethal to the dams (Baxley et al. 1981; Beaudoin 1974; Carpenter 1987; Ferm and Carpenter 1968; Ferm et al. 1971; Hanlon and Ferm 1986; Holson et al. 1999, 2000; Hood and Bishop 1972; Hood and Harrison 1982; Hood et al. 1978; Mason et al. 1989; Nagymajtényi et al. 1985; Nemec et al. 1998; Stump et al. 1999; Willhite 1981). There are also some data to suggest that it may increase the risk of transplacental cancer in humans (Smith et al. 2006) and animals (Waalkes et al. 2003). Thus, additional studies in animals may be useful in defining the mechanisms of these developmental effects and in identifying the time of maximum susceptibility of the fetus, but such studies probably will not help identify a safe exposure level for humans.

Organic Arsenicals. No information was located regarding developmental effects in humans after oral or inhalation exposure to organic arsenicals. Animal studies conducted in rats (Chernoff et al. 1990; Irvine et al. 2006; Rogers et al. 1981), mice (Kavlock et al. 1985; Rogers et al. 1981), and rabbits (Irvine et al. 2006) have examined the developmental toxicity of organic arsenicals. Decreases in fetal body weights and delays in ossification were commonly reported at maternally toxic (decreases in body weight gain) doses of DMA (Irvine et al. 2006; Kavlock et al. 1985; Rogers et al. 1981). However, one study found increases in the percentage of fetuses with irregular palatine rugae at DMA doses not associated with maternal toxicity (Rogers et al. 1981). This effect has not been reported in other studies and additional developmental studies are needed to confirm the finding. In view of the apparent differences in susceptibility between animals and humans, it would be valuable to investigate whether there are any measurable effects on development in humans exposed to organic arsenicals in the workplace or the environment.

Immunotoxicity.

Inorganic Arsenicals. No studies were located on immunotoxic effects in humans after oral exposure to inorganic arsenic. One inhalation study in humans (Bencko et al. 1988), an inhalation study in animals (Aranyi et al. 1985), one oral study in animals (Kerkvliet et al. 1980), and one intratracheal instillation study in animals (Sikorski et al. 1989) suggest that arsenic causes little or no functional impairment of the

immune system, but one inhalation study in animals found decreased pulmonary bactericidal activity and increased susceptibility to streptococcal infection in exposed mice (Aranyi et al. 1985). Additional studies (both in humans and animals) would be valuable to investigate this end point further. Dermal exposure of humans to high levels of arsenic dusts may cause dermal sensitization (Holmqvist 1951), but the dose and time dependence of this phenomenon are not known. Studies to determine whether dermal sensitization occurs in people with low level dermal exposures to arsenic in dust or soil, such as might occur for residents near an arsenic-containing waste site, would be valuable in assessing the significance of this effect to nonoccupationally exposed populations.

Organic Arsenicals. No information was located on the effect of organic arsenicals exposure in humans or animals on immune function. Since there are suggestions that inorganic arsenic may cause some changes in the immune system, studies on possible immune effects of the common organic arsenicals might be helpful.

Neurotoxicity.

Inorganic Arsenicals. There is convincing evidence from studies in humans that inorganic arsenic can cause serious neurological effects, both after inhalation (Beckett et al. 1986; Bencko et al. 1977; Blom et al. 1985; Buchancová et al. 1998; Calderón et al. 2001; Danan et al. 1984; Feldman et al. 1979; Gerr et al. 2000; Lagerkvist and Zetterlund 1994; Morton and Caron 1989) and oral exposure (Armstrong et al. 1984; Bartolome et al. 1999; Chakraborti et al. 2003a, 2003b; Civantos et al. 1995; Cullen et al. 1995; Danan et al. 1984; EPA 1977a; Feldman et al. 1979; Fincher and Foy et al. 1992; Franzblau and Lilis 1989; Guha Mazumder et al. 1988; Hindmarsh et al. 1977; Huang et al. 1985; Fincher and Koerker 1987; Levin-Scherz et al. 1987; Lewis et al. 1999; Mizuta et al. 1956; Muzi et al. 2001; Quatrehomme et al. 1992; Silver and Wainman 1952; Szuler et al. 1979; Tsai et al. 2003; Uede and Furukawa 2003; Vantroyen et al. 2004; Wagner et al. 1979). This is based mainly on clinical observations and neurological examinations of exposed persons. Available studies provide a reasonable estimate of LOAEL and NOAEL values by the oral route, but similar data are lacking for the inhalation route. Further studies designed to identify the threshold for neurological effects in humans exposed by the inhalation route would be valuable, since humans may be exposed to arsenic dusts in air from smelters, chemical factories, or waste sites. Adult animals appear to be much less susceptible than humans to the neurological effects of inorganic arsenic, so studies in adult animals would probably not help in estimation of a safe exposure limit. However, in light of recent findings of possible associations between arsenic in drinking water and neurobehavioral alterations in children (Tsai et al. 2003; von Ehrenstein et

al. 2007; Wang et al. 2007; Wasserman et al. 2004, 2007), studies in animals, in which confounding can be eliminated, may be warranted.

Organic Arsenicals. Information on neurological effects of organic arsenicals in humans is limited to an occupational study that did not find increases in the frequency of central or peripheral nervous system complaints (Watrous and McCaughey 1945) and a case report of a women of a women reporting numbness and tingling of the fingertips, toes, and circomoral region who was exposed to organic arsenic in soup (Luong and Nguyen 1999). Neurological effects have also been observed in some animal studies. Decreases spontaneous motility, ataxia, and increased startle response were observed in mice exposed to a single high dose of DMA (Kaise et al. 1989). Degeneration of myelin and axons were observed in several studies involving oral exposure of pigs to roxarsone (Edmonds and Baker 1986; Kennedy et al. 1986; Rice et al. 1985). Hyperexcitability, ataxia, and trembling have also been observed in rats and mice orally exposed to roxarsone (Kerr et al. 1963; NTP 1989b). These findings suggest that more extensive investigations of the neurotoxic potential of roxarsone and other organic arsenicals would be valuable to determine the potential human health risk from these compounds, since humans could be exposed during the manufacture or use of these compounds, or near waste sites where they have been deposited.

Epidemiological and Human Dosimetry Studies. Numerous epidemiologic studies of humans exposed to inorganic arsenic by the oral and inhalation routes constitute the database on arsenic-related cancer and noncancer human health effects. As with virtually all epidemiologic investigations, these studies are limited by possible confounding from factors such as smoking, exposure to other chemicals, and differences in population characteristics (e.g., nutritional state, metabolism, and toxicokinetics) that inhibit extrapolation of study results to a wider population. Moreover, many of these studies lack good dose estimates for study participants. Some studies lack quantitative data altogether. For this reason, improved data on confounding factors and improved methods of human dosimetry would be valuable in any further human epidemiologic studies of arsenic, either in the workplace or in the general environment. Recent work has broadened the qualitative dose-response information beyond the highly exposed Taiwanese population, but additional studies of persons with lower exposure levels would be especially valuable for risk assessments for the U.S. population. From a public health standpoint, well designed studies of common noncancer health outcomes (e.g., cardiovascular disease and diabetes) could be more important than additional studies of cancer. Availability of methods for biomonitoring of exposure are discussed below.

Biomarkers of Exposure and Effect.

Exposure. There are sensitive and specific methods for measuring arsenic in blood, urine, hair, nails, and other tissues, and this is the approach normally employed for measuring arsenic exposure in humans. Usually total arsenic is measured, but methods are available for measuring inorganic arsenic and each of the organic derivatives separately. Urinary levels are generally considered to be the most reliable indication of recent exposures (Enterline et al. 1987a; Milham and Strong 1974; Pinto et al. 1976; Polissar et al. 1990), but if a high urinary level is present, care must be taken to account for the presence of nontoxic forms of arsenic from the diet. Blood levels are sometimes used to evaluate the status of acutely poisoned individuals (Driesback 1980; Heydorn 1970; Hindmarsh and McCurdy 1986; Valentine et al. 1979, 1981), but this approach is not generally useful for biomonitoring of long-term exposure to low levels. Hair and nails provide a valuable indication of exposures that occurred 1–10 months earlier (Agahian et al. 1990; Bencko et al. 1986; Choucair and Ajax 1988; EPA 1977a, 1981b; Milham and Strong 1974; Valentine et al. 1979; Yamauchi et al. 1989), although care must be taken to exclude external contamination of these samples. Cumulative urinary arsenic levels may be used to derive a quantitative estimate of exposure (Enterline et al. 1987a; Pinto et al. 1976), but data on the quantitative relation between exposure and arsenic levels in nails and hair were not located. Efforts to establish an algorithm for estimating past exposure levels from hair or nail levels would be valuable in quantifying average long-term exposure levels in people where repeated urinary monitoring is not feasible.

Effect. The effects of arsenic are mainly nonspecific, but the combined presence of several of the most characteristic clinical signs (e.g., nausea, diarrhea, peripheral neuropathy, anemia, vascular lesions, hyperkeratinization, hyperpigmentation) is usually adequate to suggest arsenic intoxication. Although there are standard clinical methods for detecting and evaluating each of these effects, there are no recognized methods for identifying early (preclinical) effects in exposed persons. Neurophysiological measurements of nerve conduction velocity or amplitude have been investigated (Goebel et al. 1990; Jenkins 1966; Le Quesne and McLeod 1977; Morton and Caron 1989; Murphy et al. 1981), but at present, this approach does not seem to offer much advantage over a standard neurological examination. Changes in urinary excretion levels of several heme-related metabolites appear to be a good indication of preclinical effects of arsenic toxicity in animals (Albores et al. 1989; Sardana et al. 1981; Woods and Fowler 1978; Woods and Southern 1989), but this has not been established in humans and is not specific for arsenic-induced effects. Further efforts to develop these approaches and to identify other more specific biochemical or physiological indicators of arsenic-induced effects would be very valuable in monitoring the health of persons exposed to low levels of arsenic in the environment or near waste sites.

Absorption, Distribution, Metabolism, and Excretion. Available data from toxicokinetic studies in humans reveal that arsenates and arsenites are well absorbed following both oral and inhalation exposure. Data on distribution are limited, but it appears that arsenic is transported to nearly all tissues. Metabolism involves mainly reduction-oxidation reactions that interconvert As(+5) and As(+3) and methylation of As(+3) to yield MMA and DMA. Most arsenic is rapidly excreted in the urine as a mixture of inorganic arsenics, MMA, and DMA, although some may remain bound in tissues (especially skin, hair, and fingernails). These findings are strongly supported by numerous studies in animals. Because methylation represents a detoxification pathway, an area of special interest is the capacity of the human body to methylate inorganic arsenic. Limited data suggest that the methylation system might begin to become saturated at intakes of about 0.2–1 mg As/day (Buchet et al. 1981b; Marcus and Rispin 1988), but this is uncertain. Further studies to define the rate and saturation kinetics of whole-body methylation in humans would be especially helpful in evaluating human health risk from the low levels of arsenic intake that are usually encountered in the environment. Along the same line, further studies to determine the nature and magnitude of individual variations in methylation capacity and how this depends on diet, age, and other factors would be very useful in understanding and predicting which members of a population are likely to be most susceptible.

The toxicokinetics of dermal exposure have not been studied. It is usually considered that dermal uptake of arsenates and arsenites is sufficiently slow that this route is unlikely to be of health concern (except that due to direct irritation), but studies to test the validity of this assumption would be valuable. Also, dermal uptake of organic arsenicals could be of concern, and quantitative data on the rate and extent of this would be helpful in evaluating risks from application of arsenical pesticides or exposures to organic arsenicals in waste sites.

Comparative Toxicokinetics. Available toxicity data indicate that arsenic causes many of the same effects in animals that are observed in humans, but that animals are significantly less sensitive. The basis for this difference in susceptibility is not certain but is probably mainly a result of differences in absorption, distribution, metabolism, or excretion. For example, rats strongly retain arsenic in red blood cells (Lanz et al. 1950), while humans (and most other species) do not. Similarly, marmoset monkeys do not methylate inorganic arsenic (Vahter and Marafante 1985; Vahter et al. 1982), while humans and other animal species do. Because of these clear differences in toxicity and toxicokinetics between species, further comparative toxicokinetic studies that focus on the mechanistic basis for these differences would be very valuable. At a minimum, this would help clarify which laboratory species are the most useful

models for humans and could ultimately lead to development of a PBPK model that would permit reliable extrapolation of observations across species.

Methods for Reducing Toxic Effects. There are a number of general methods for reducing the absorption of arsenic in the gastrointestinal tract and skin, but there are currently no methods for reducing the absorption of arsenic from the lungs. The removal of arsenic from the gastrointestinal tract is usually facilitated by the use of emetics, cathartics, lavages, or activated charcoal (Agency for Toxic Substances and Disease Registry 1990a; Aposhian and Aposhian 1989; Campbell and Alvarez 1989; Driesback 1980; Ellenhorn and Barceloux 1988; EPA 1989e; Haddad and Winchester 1990; Mitra et al. 2004; Stutz and Janusz 1988). Studies that investigate the effects of phosphate-binding chemicals (aluminum hydroxide) and nonabsorbable sulfhydryl compounds on the absorption of pentavalent and trivalent arsenic, respectively, may be useful in developing treatments that are more specific to arsenic intoxication. Once arsenic is in the body, treatment usually involves the use of one or more chelators, such as BAL or penicillamine. However, these agents often exhibit adverse side effects (Agency for Toxic Substances and Disease Registry 1990a; Ellenhorn and Barceloux 1988), and are generally only applied following high-dose acute exposure. Further studies investigating the efficacy of less toxic arsenic chelators, such as DMSA, DMPA, DMPS, and N-acetyl cysteine, may lead to the development of safer treatment methods. Studies on the efficacy of chelators and agents to enhance methylation and elimination in treatment of chronic arsenic exposure would also be helpful, as available treatment methods for chronic arsenic exposure are limited. Trivalent arsenic is generally believed to exert toxic effects by binding to the vicinal sulfhydryl group of key enzymes, thereby interfering with a number of biological processes, such as gluconeogenesis and DNA repair (Li and Rossman 1989; Szinicz and Forth 1988). Since pentavalent arsenic may need to be reduced in the body to the trivalent state before it can exert toxic effects, studies that investigate methods for blocking this conversion may lead to a method for interfering with the mechanism of action for pentavalent arsenic. The insufficient intake of calcium, animal protein, folate, selenium, and fiber may enhance the toxic effects of inorganic arsenic (Mitra et al. 2004), but it is not known if dietary supplementation will prove effective in patients who already show arsenic-induced symptoms.

Children's Susceptibility. Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection above.

A majority of the data on the effects of exposure of humans to arsenic has focused on adults. Although a few studies of acute poisoning and chronic exposure specifically describe children (Borgoño et al. 1980; Concha et al. 1998a, 1998b, 1999; Foy et al. 1992; Kersjes et al. 1987; Rosenberg 1974; Zaldívar 1974; Zaldívar and Guillier 1977), in general, data are lacking. Specifically, although there is a substantial database on the effect of arsenic on animal development, there are few data describing developmental effects in humans. Additional research in this area, using populations in areas of endemic arsenic exposure, would be useful.

Although there is no reason to suspect that the pharmacokinetics of arsenic differs in children and adults, there are few data available on this topic. Research on absorption, distribution, metabolism, and excretion in children would aid in determining if children are at an increased risk, especially in areas where chronic exposure to an environmental source occurs. With regard to exposure during development, additional research on maternal kinetics, and transfer via breast milk would be useful in obtaining a more complete picture of prenatal and neonatal development, especially with regard to neural development and the possible development of childhood cancer.

Child health data needs relating to exposure are discussed in Section 6.8.1, Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

A number of researchers are continuing to investigate the toxicity and toxicokinetics of arsenic. Table 3-18 summarizes studies being sponsored by agencies of the U.S. federal government (FEDRIP 2007). Additional research is being sponsored by industry groups and other agencies, and research is also ongoing in a number of foreign countries.

Table 3-18. Ongoing Studies on Health Effects of Arsenic, Federally Funded

Investigator	Affiliation	Title	Sponsor
Ahsan, H	Columbia University, New York, New York	Chemoprevention of arsenic-induced skin cancer	NCI
Ahsan, H	Columbia University, New York, New York	Genetic susceptibility to arsenic-induced skin cancer	NCI
Andrew, A	Darmouth College, Hanover, New Hampshire	Bladder cancer prognostic indicators	NCI
Beckman, K	Children's Hospital and Research Center, Oakland, California	Fetal arsenic-nutrient interaction in adult-onset cancer	NIEHS
Bodwell, J	Darmouth College, Hanover, New Hampshire	Arsenic effects on glucocorticoid receptor action	NIEHS
Calderon, R	EPA, Research Triangle Park, North Carolina	Arsenic-induced skin conditions identified in Southwest United States	HEERL
Christiani, D	Harvard University, Boston, Massachusetts	Arsenic and health in Bangladesh	NIEHS
Dong, Z	University of Minnesota, Minneapolis, Minnesota	Molecular basis of arsenic-induced cell transformation	NCI
Finnell, R	Texas A & M University College Station, Texas	Sensitive genotypes to arsenic as a model environmental toxicant	NIEHS
Frenkel, K	New York University, New York, New York	Metal induced inflammatory factors, oxidative stress, and suppression	NIEHS
Futscher, B	University of Arizona, Tucson, Arizona	Epigenetic remodeling by environmental arsenicals	NCI
Gamble, M	Columbia University, New York, New York	Nutritional influences on arsenic toxicity	NIEHS
Germolec, D	NIH, Research Triangle Park, North Carolina	The role of growth factors and inflammatory mediators in arsenic-induced dermatotoxicity	NIEHS
Guallar, E	Johns Hopkins University, Baltimore, Maryland	Mercury, arsenic, and carotid atherosclerosis	NIEHS
He, K	Northwestern University, Chicago, Illinois	Trace elements and CVD risks factors among young adults	NHLBI
Hei, T	Columbia University, New York, New York	Mechanisms of arsenic carcinogenesis	NIEHS
Huang, C	New York University, New York, New York	Effects of arsenic on PI-3K signaling pathway	NCI
Hudgens, E	EPA, Research Triangle Park, North Carolina	Study of individuals chronically exposed to arsenic in drinking water	HEERL
Hughes, M	EPA, Research Triangle Park, North Carolina	Biomarkers of exposure: a case study with inorganic arsenic	HEERL
Hughes, M	EPA, Research Triangle Park, North Carolina	Tissue dosimetry, metabolism, and excretion of pentavalent arsenic	HEERL
Jing, Y	New York University, New York, New York	Arsenic trioxide and acute myeloid leukemia	NCI

Table 3-18. Ongoing Studies on Health Effects of Arsenic, Federally Funded

Investigator	Affiliation	Title	Sponsor
Jung, M	Georgetown University, Washington, DC	Epigenetic regulation by poly(ADP-ribose) in response to arsenite	NIEHS
Karin, M	University of California San Diego, La Jolla, California	Interaction of heavy metal ions with the human genome	NIEHS
Kelsey, K	Harvard University, Boston, Massachusetts	Arsenic mode of action in cancer—models of epigenetic mechanism	NIEHS
Liu, K	University of New Mexico, Albuquerque, New Mexico	Oxidative mechanisms of arsenic-induced carcinogenesis	NIEHS
Markowski, V	University of Southern Maine, Portland, Maine	Developmental arsenic exposure produces cognitive impairment	NIEHS
Martin, M	Georgetown University, Washington, DC	Arsenic and epigenetic regulation of gene expression	NIEHS
Muscarella, D	Cornell University Ithaca, Ithaca, New York	Arsenite effects on CD40 signaling and B-cell apoptosis	NIEHS
Nichols, R	Dartmouth College, Hanover, New Hampshire	Effect of arsenic on cytochrome P450	NIEHS
Nriagu, J	University of Michigan, Ann Harbor, Michigan	Arsenic exposure and bladder cancer in Michigan	NCI
Rosen, B	Wayne State University, Detroit, Michigan	Mechanisms of arsenical transport	NIGMS
Rosen, B	Wayne State University, Detroit, Michigan	Metal binding domains in metallo-regulatory proteins	NIAID
Rosenblatt, A	University of Miami, Coral Gables, Florida	Environmental arsenic and androgen receptor regulation	NIEHS
Rossman, T	New York University, New York, New York	Investigation and genetic analysis of the human arsenite efflux pump	NIEHS
Schwartz, J	Harvard University, Boston Massachusetts	Epigenetic effects of particles and metals on cardiac health of an aging cohort	NIEHS
Self, W	University of Central Florida, Orlando, Florida	Impact of arsenicals on selenoprotein synthesis	NIEHS
Sens, D	University of North Dakota, Grand Forks, North Dakota	Metallothionein isoform-3 urinary marker bladder cancer	NIEHS
Sheldon, L	Dartmouth College, Hanover, New Hampshire	Arsenic, histone modification, and transcription	NIEHS
Shi, X	University of Kentucky, Lexington, Kentucky	Mechanism of arsenic-induced carcinogenesis	NCI
Smith, A	University of California, Berkeley, California	Arsenic biomarker epidemiology	NIEHS
Spallholz, J	Texas Tech University, Lubbock, Texas	Selenium against arsenic toxicity and skin lesions	NCI
States, C	University of Louisville, Louisville, Kentucky	Arsenic induced miotic arrest associated apoptosis	NIEHS
Styblo, M	University of North Carolina, Chapel Hill, North Carolina	Metabolism and toxicity of arsenic in human liver	NIEHS

Table 3-18. Ongoing Studies on Health Effects of Arsenic, Federally Funded

Investigator	Affiliation	Title	Sponsor
Taylor, P	Division of Cancer Epidemiology and Genetics, NCI, Bethesda, Maryland	Biologic specimen bank for early lung cancer markers in Chinese tin miners	NCI
Taylor, B	University of Louisville, Louisville, Kentucky	Arsenite inhibition of mitotic progression	NIEHS
Vaillancourt, R	University of Arizona, Tucson, Arizona	Modulation of Prostaglandins by Arsenic	NIEHS
Willett, W	Harvard University, Boston, Massachusetts	Prospective studies of diet and cancer in men and women	NCI
Wright, R	Brigham and Women's Hospital, Boston, Massachusetts	Metal mixtures and neurondevelopment	NIEHS
Zhang, D	University of Arizona, Tucson, Arizona	The protective role of Nrf2 in arsenic-induced toxicity and carcinogenicity	NIEHS

EPA = Environmental Protection Agency; NHEERL = National Health and Environmental Effects Research Laboratory; NCI = National Cancer Institute; NHLBI = National Heart, Lung, and Blood Institute; NIEHS = National Institute of Environmental Health Sciences; NIAID= National Institute of Allergy and Infectious Diseases; NIGMS = National Institute of General Medical Sciences; NIH = National Institute of Health

Source: FEDRIP 2007

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4. CHEMICAL AND PHYSICAL INFORMATION

4.1 CHEMICAL IDENTITY

Information regarding the chemical identity of arsenic and some common inorganic and organic arsenic compounds are located in Tables 4-1 and 4-2, respectively.

4.2 PHYSICAL AND CHEMICAL PROPERTIES

Information regarding the physical and chemical properties of arsenic and some common inorganic and organic arsenic compounds is located in Tables 4-3 and 4-4, respectively.

Arsenic appears in Group 15 (V) of the periodic table, below nitrogen and phosphorus. Arsenic is classified chemically as a metalloid, having both properties of a metal and a nonmetal; however, it is frequently referred to as a metal. Elemental arsenic, which is also referred to as metallic arsenic, (As(0)) normally occurs as the α-crystalline metallic form, which is a steel gray and brittle solid. The β-form is a dark gray amorphous solid. Other allotropic forms of arsenic may also exist. In compounds, arsenic typically exists in one of three oxidation states, -3, +3, and +5 (Carapella 1992). Arsenic compounds can be categorized as inorganic, compounds without an arsenic-carbon bond, and organic, compounds with an arsenic-carbon bond.

Table 4-1. Chemical Identity of Arsenic and Selected Inorganic Arsenic Compounds[a]

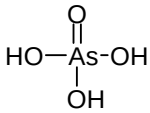
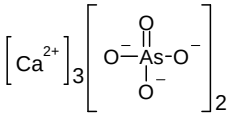
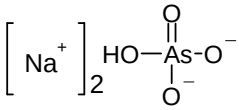
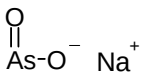
Characteristic	Arsenic	Arsenic acid	Arsenic pentoxide	Arsenic trioxide
Synonym(s)	Arsenic black; colloidal arsenic; gray arsenic, metallic arsenic	Orthoarsenic acid	Arsenic(V) oxide; arsenic acid anhydride; diarsenic pentoxide	Arsenic(III) oxide; arsenious acid; arsenious oxide; white arsenic
Registered trade name(s)	No data	Zotox; Hi-Yield Desiccant H-10; Desiccant L-10; Crab Grass Killer	No data	White Arsenic; Arsenicum Album
Chemical formula	As	H_3AsO_4	As_2O_5	As_2O_3
Chemical structure	As		$[As^{5+}]_2\ [O^{2-}]_5$	$[As^{3+}]_2\ [O^{2-}]_3$
Identification numbers:				
CAS registry	7440-38-2	7778-39-4	1303-28-2	1327-53-3
NIOSH RTECS[b]	CG0525000	CG0700000	CG2275000	CG3325000
EPA hazardous waste	D004	D004, P010	D004, P011	D004, P012
OHM/TADS	No data	No data	No data	No data
DOT/UN/NA/IMDG shipping	UN1558/IMDG 6.1	UN1553 (liquid)/ UN1554 (solid)/ IMDG 6.1 (liquid and solid)	UN1559/IMDG 6.1	UN1561/ IMDG 6.1
HSDB	509	431	429	419
EINECS	231-148-6	231-901-9	215-116-9	215-481-4
NCI	No data	No data	No data	No data

Table 4-1. Chemical Identity of Arsenic and Selected Inorganic Arsenic Compounds[a]

Characteristic	Calcium arsenate	Gallium arsenide	Sodium arsenate	Sodium arsenite
Synonym(s)	Calcium ortho-arsenate; arsenic acid, calcium salt	Gallium mono-arsenide	Disodium arsenate, dibasic; disodium hydrogen arsenate; arsenic acid, disodium salt	Arsenenous acid, sodium salt; sodium meta-arsenite
Registered trade name(s)	Pencal; Security; Turf-Cal; Chip-Cal; SPRA-Cal	No data	No data	Atlas "A"; Penite; Kill-All; Chem-Sen 56; Chem Pels C; Progalumnol Double
Chemical formula	$Ca_3(AsO_4)_2$	GaAs	Na_2HAsO_4	$NaAsO_2$
Chemical structure		Ga:As		
Identification numbers:				
CAS registry	7778-44-1	1303-00-0	7778-43-0	7784-46-5
NIOSH RTECS[b]	CG0830000	LW8800000	CG0875000	CG3675000
EPA hazardous waste	D004	D004	D004	D004
OHM/TADS	No data	No data	No data	7800057
DOT/UN/NA/IMDG shipping	UN1573/IMDG 6.1	UN 2803; Gallium/ IMDG 8.0; Gallium	UN 1685/IMDG 6.1	UN1686 (aqueous solution)/UN2027 (solid)/IMDG 6.1
HSDB	1433	4376	1675	693
EINECS	233-287-8	215-114-8	231-902-4	232-070-5
NCI	No data	No data	No data	No data

[a]All information obtained from HSDB 2007 and CHEMIDplus 2007, except where noted.
[b]RTECS 2007

CAS = Chemical Abstracts Service; DOT/UN/NA/IMDG = Department of Transportation/United Nations/North America/Intergovernmental Maritime Dangerous Goods Code; EINECS = European Inventory of Existing Chemical Substances; EPA = Environmental Protection Agency; HSDB = Hazardous Substances Data Bank; NCI = National Cancer Institute; NIOSH = National Institute for Occupational Safety and Health; OHM/TADS = Oil and Hazardous Materials/Technical Assistance Data System; RTECS = Registry of Toxic Effects of Chemical Substances

Table 4-2. Chemical Identity of Selected Organic Arsenic Compounds[a]

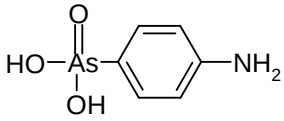
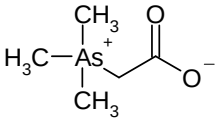
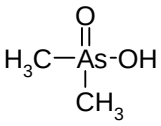
Characteristic	Arsanilic acid	Arsenobetaine	Dimethylarsinic acid
Synonym(s)	(4-Aminophenyl)arsonic acid; antoxylic acid; atoxylic acid, Pro-Gen	Arsonium, (carboxymethyl)-trimethyl-, hydroxide, inner salt	Cacodylic acid; hydroxydi-methyl-arsine oxide; DMA; DMAA
Registered trade name(s)	No data	No data	510; Arsan; Phytar 560; Rad-E-Cate 35
Chemical formula	$C_6H_8AsNO_3$	$C_5H_{11}AsO_2$	$C_2H_7AsO_2$
Chemical structure			
Identification numbers:			
CAS registry	98-50-0	64436-13-1	75-60-5
NIOSH RTECS[b]	CF7875000	CH9750000	CH7525000
EPA hazardous waste	D004	No data	U136/D004
OHM/TADS	No data	No data	No data
DOT/UN/NA/IMDG shipping	No data	No data	UN1572/IMDG 6.1
HSDB	432	No data	360
EINECS	202-674-3	No data	200-883-4
NCI	No data	No data	No data

Table 4-2. Chemical Identity of Selected Organic Arsenic Compounds[a]

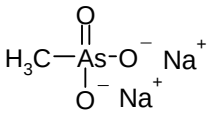
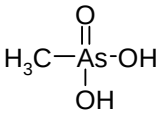
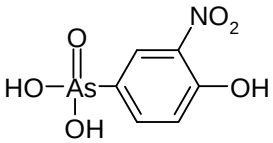
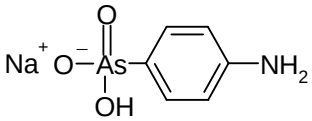
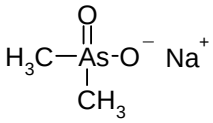
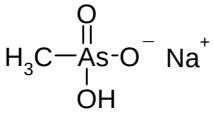
Characteristic	Disodium methane-arsonate	Methanearsonic acid	3-Nitro-4-hydroxy-phenyl-arsonic acid
Synonym(s)	DSMA; disodium monomethane arsonate	Arsonic acid, methyl-; monomethylarsonic acid	Roxarsone; 3-nitro-4-hydroxyphenylarsonic acid; 3-Nitro-10
Registered trade name(s)	Ansar 8100; Arrhenal; Ansar DSMA Liquid; Dinate; Crab-E-Rad; Chipco Crab Kleen; Arsinyl; Sodar; Methar; Drexel DSMA Liquid; Di-Tac; Ansar 184; Weed-E-Rad; Versar DSMA-LQ; Calar-E-Rad; Dal-E-Rad; Jon-Trol; Namate	No data	No data
Chemical formula	$CH_3AsO_3Na_2$	CH_5AsO_3	$C_6H_6AsNO_6$
Chemical structure			
Identification numbers:			
CAS registry	144-21-8	124-58-3	121-19-7
NIOSH RTECS[b]	PA2275000	PA1575000	CY5250000
EPA hazardous waste	D004	D004	D004
OHM/TADS	No data	No data	No data
DOT/UN/NA/IMDG shipping	No data	No data	No data
HSDB	1701	845	4296
EINECS	205-620-7	204-705-6	204-453-7
NCI	No data	No data	C5608

Table 4-2. Chemical Identity of Selected Organic Arsenic Compounds[a]

Characteristic	Sodium arsanilate	Sodium dimethylarsinate	Sodium methanearsonate
Synonym(s)	(4-Aminophenyl)arsonic acid sodium salt; arsanilic acid sodium salt; arsamin; atoxyl; soamin; trypoxyl	Sodium cacodylate; cacodylic acid, sodium salt; sodium dimethylarsonate	Arsonic acid, methyl-, monosodium salt; monosodium acid metharsonate; MSMA
Registered trade name(s)	No data	Ansar 160; Ansar 560; Bolls-Eye; Chemaid; Phytar 560, component of (with 012501); Rad-E-Cate 25.	Ansar 529; Ansar 170; Target MSMA; Phyban H.C.; Deconate; Mesamate; Bueno; Monate Merge 823; Dal-E-Rad; Weed-S-Rad; Arsanote liquid; Silvisar 550.
Chemical formula	$C_6H_7AsNO_3Na$	$C_2H_6AsO_2Na$	CH_4AsO_3Na
Chemical structure			
Identification numbers:			
CAS registry	127-85-5	124-65-2	2163-80-6
NIOSH RTECS[b]	CF9625000	CH7700000	PA2625000
EPA hazardous waste	D004	D004	D004
OHM/TADS	No data	No data	No data
DOT/UN/NA/IMDG shipping	UN2473/IMDG 6.1	UN1688/IMDG 6.1	No data
HSDB	5189	731	754
EINECS	204-869-9	204-708-2	218-495-9
NCI	C61176	No data	C60071

[a]All information obtained from HSDB 2007 and CHEMIDplus 2007, except where noted.
[b]RTECS 2007

CAS = Chemical Abstracts Service; DOT/UN/NA/IMDG = Dept. of Transportation/United Nations/North America/Intergovernmental Maritime Dangerous Goods Code; EINECS = European Inventory of Existing Chemical Substances; EPA = Environmental Protection Agency; HSDB = Hazardous Substances Data Bank; NCI = National Cancer Institute; NIOSH = National Institute for Occupational Safety and Health; OHM/TADS = Oil and Hazardous Materials/Technical Assistance Data System; RTECS = Registry of Toxic Effects of Chemical Substances

Table 4-3. Physical and Chemical Properties of Arsenic and Selected Inorganic Arsenic Compounds[a]

Property	Arsenic	Arsenic acid	Arsenic pentoxide	Arsenic trioxide
Molecular weight	74.9216	141.944	229.840	197.841
Color	Silver-gray or tin-white	White[b]	White	White
Physical state	Solid	Solid[b]	Solid	Solid
Melting point	817 °C (triple point)	35 °C	Decomposes at ~300 °C	313 °C (claudetite) 274 °C (arsenolite)
Boiling point	614 °C sublimes	Loses H_2O at 160 °C [b]	No data	460 °C
Density	5.778 g/cm^3 at 25 °C	~2.2 g/cm^3	4.32 g/cm^3	3.865 g/cm^3 (cubes) 4.15 g/cm^3 (rhombic crystals)
Odor	Odorless	No data	No data	Odorless
Odor threshold:				
Water	No data	No data	No data	No data
Air	No data	No data	No data	No data
Solubility:				
Water	Insoluble	302 g/L at 12.5 °C[b]	2,300 g/L at 20 °C	17 g/L at 16 °C
Organic solvent(s)	No data	Soluble in alcohol, glycerol[b]	Soluble in alcohol	Practically insoluble in alcohol, chloroform, ether; soluble in glycerol
Other	Insoluble in caustic and nonoxidizing acids	No data	Soluble in acid, alkali	Soluble in dilute hydrochloric acid, alkali hydroxide, carbonate solution
Partition coefficients:				
Log K_{ow}	No data	No data	No data	No data
Log K_{oc}	No data	No data	No data	No data
pK_a	No data	pK_{a1}=2.22; pK_{a2}=6.98 pK_{a3}=11.53[c]	No data	No data
Vapor pressure	7.5x10^{-3} mmHg at 280 °C	No data	No data	2.47x10^{-4} mmHg at 25 °C
Autoignition temperature	No data	No data	No data	Not flammable
Flashpoint	No data	No data	No data	No data
Flammability limits in air	No data	No data	No data	No data
Conversion factors	No data	No data	No data	No data
Explosive limits	No data	No data	No data	No data

Table 4-3. Physical and Chemical Properties of Arsenic and Selected Inorganic Arsenic Compounds[a]

Property	Calcium arsenate	Gallium arsenide	Disodium arsenate	Sodium arsenite
Molecular weight	398.072	144.64	185.91	130.92
Color	Colorless	Dark gray	Colorless[d]	White to gray-white
Physical state	Solid	Solid	Solid[d]	Solid
Melting point	Decomposes on heating	1,238 °C	57 °C[d]	No data
Boiling point	No data	No data	No data	No data
Density	3.620 g/cm^3	5.3176 g/cm^3 at 25 °C	1.87 g/cm[d]	1.87 g/cm^3
Odor	Odorless	Garlic odor	Odorless[d]	No data
Odor threshold:				
Water	No data	No data	No data	No data
Air	No data	No data	No data	No data
Solubility:				
Water	0.13 g/L at 25 °C	<1 mg/mL at 20 °C	Soluble 1:3 parts in water[d]	Freely soluble in water
Organic solvents	Insoluble	<1 mg/mg dimethyl sulfoxide, ethanol, methanol, acetone	Slightly soluble in alcohol; soluble in glycerol[d]	Slightly soluble in alcohol
Other	Soluble in dilute acids	Soluble in hydrochloric acid	Slightly soluble in alkaline solution[d]	No data
Partition coefficients:				
Log K_{ow}	No data	No data	No data	No data
Log K_{oc}	No data	No data	No data	No data
pK_a	No data	No data		
Vapor pressure	~0 mmHg at 20 °C	No data	No data	No data
Autoignition temperature	Not combustible	No data	No data	Not combustible
Flashpoint	No data	No data	No data	No data
Flammability limits in air	No data	No data	No data	No data
Conversion factors	No data	No data	No data	No data
Explosive limits	No data	No data	No data	No data

[a]All information from HSDB 2007, except where noted.
[b]Value for arsenic acid hemihydrate
[c]NRC 1999
[d]Value for disodium arsenate heptahydrate

Table 4-4. Physical and Chemical Properties of Selected Organic Arsenic Compounds[a]

Property	Arsenilic acid	Arsenobetaine	Dimethylarsinic acid
Molecular weight	217.06	196.1[b]	138.00
Color	White	No data	Colorless
Physical state	Solid	Solid[b]	Solid
Melting point	232 °C	203–210 °C (decomposes)[b]	195 °C
Boiling point		No data	>200 °C
Density	1.9571 g/cm^3 at 10 °C	No data	No data
Odor	Practically odorless	No data	Odorless
Odor threshold:			
Water	No data	No data	No data
Air	No data	No data	No data
Solubility:			
Water	Slightly soluble in cold water; soluble in hot water	No data	2,000 g/L at 25 °C
Organic solvent(s)	Slightly soluble in alcohol; soluble in amyl alcohol; insoluble in ether, acetone, benzene, chloroform	No data	Soluble in alcohol; insoluble in diethyl ether
Acids	Slightly soluble in acetic acid; soluble in alkaki carbonates; moderately soluble in concentrated mineral acids; insoluble in dilute mineral acids	No data	Soluble in acetic acid
Partition coefficients:			
Log K_{ow}	No data	No data	No data
Log K_{oc}	No data	No data	No data
pK_a	No data	2.2[c]	1.57
Vapor pressure	No data	No data	No data
Henry's law constant	No data	No data	No data
Autoignition temperature	No data	No data	No data
Flashpoint	No data	No data	No data
Flammability	No data	No data	Nonflammable
Conversion factors	No data	No data	No data
Explosive limits	No data	No data	No data

Table 4-4. Physical and Chemical Properties of Selected Organic Arsenic Compounds[a]

Property	Methanearsonic acid	3-Nitro-4-hydroxy-phenylarsonic acid	Sodium arsanilate
Molecular weight	139.97	263.03	239.04
Color	White	Pale yellow	White or creamy white
Physical state	Solid	Solid	Solid
Melting point	160.5 °C	No data	No data
Boiling point	No data	No data	No data
Density	No data	No data	No data
Odor	No data	No data	Odorless
Odor threshold:			
Water	No data	No data	No data
Air	No data	No data	No data
Solubility:			
Water	256 g/L at 20 °C	Slightly soluble in cold water; soluble in about 30 parts boiling water	Soluble 1 part in 3 parts water
Organic solvents	Soluble in ethanol	Soluble in methanol, ethanol, acetone; insoluble in ether, ethyl acetate	Soluble 1 part in 150 parts alcohol; practically insoluble in chloroform, ether
Acids	No data	Soluble in acetic acid, alkalies; sparingly soluble in dilute mineral acids	No data
Partition coefficients:			
Log K_{ow}	No data	No data	No data
Log K_{oc}	No data	No data	No data
pK_a	pK_{a1}=4.1; pK_{a2}=9.02	No data	No data
Vapor pressure at 25 °C	$<7.5 \times 10^{-8}$ mmHg	No data	No data
Henry's law constant	No data	No data	No data
Autoignition temperature	No data	No data	No data
Flashpoint	No data	No data	No data
Flammability	No data	No data	No data
Conversion factors	No data	No data	No data
Explosive limits	No data	No data	No data

Table 4-4. Physical and Chemical Properties of Selected Organic Arsenic Compounds[a]

Property	Disodium methanearsonate	Sodium dimethylarsinate	Sodium methanearsonate
Molecular weight	183.93	159.98	161.95
Color	White	Colorless to light yellow	White
Physical state	Solid	Solid	Solid
Melting point	>355 °C	200 °C	130–140 °C
Boiling point	No data	No data	No data
Density	1.04 g/cm^3	>1 g/cm^3 at 20 °C	1.55 g/mL[d]
Odor	No data	Odorless	Odorless
Odor threshold:			
Water	No data	No data	No data
Air	No data	No data	No data
Solubility:			
Water	432 g/L at 25 °C	200 g/L at 25 °C	580 g/L at 20 °C
Organic solvents	Soluble in methanol; practically insoluble in most organic solvents	No data	Insoluble in most organic solvents
Acids	No data	No data	No data
Partition coefficients:			
Log K_{ow}	<1	No data	-3.10
Log K_{oc}	No data	No data	No data
pK_a	pK_{a1}=4.1; pK_{a2}=8.94	6.29	pK_{a1}=4.1; pK_{a2}=9.02
Vapor pressure at 25 °C	10^{-7} mmHg	No data	7.8×10^{-8} mmHg
Henry's law constant	No data	No data	No data
Autoignition temperature	No data	No data	No data
Flashpoint	No data	No data	No data
Flammability	Nonflammable	No data	Nonflammable
Conversion factors	No data	No data	No data
Explosive limits	No data	No data	No data

[a]All information from HSDB 2007, except where noted.
[b]Cannon et al. 1981 (arsenobetaine as monohydrate)
[c]Teräsahde et al. 1996
[d]Value for Ansar 6.6

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5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

5.1 PRODUCTION

Arsenic is presently obtained as a byproduct of the smelting of copper, lead, cobalt, and gold ores. Arsenic trioxide is volatilized during smelting and accumulates in the flue dust, which may contain up to 30% arsenic trioxide. The crude flue dust is further refined by mixing with small amounts of galena or pyrite to prevent the formation of arsensites and roasting to yield arsenic trioxide of 90–95% purity. By successive sublimations, a purity of 99% can be obtained. Elemental arsenic can be prepared by the reduction of arsenic oxide with charcoal. Demand for elemental arsenic is limited and thus, about 95% of arsenic is marketed and consumed in combined form, principally as arsenic trioxide, which is subsequently converted to arsenic acid (Carapella 1992; Hanusch et al. 1985; USGS 2006a).

Since 1985, when the ASARCO smelter in Tacoma, Washington ceased operation, there has been no domestic production of arsenic trioxide or elemental arsenic and consequently, the United States remains entirely dependent on imports (U.S. Bureau of Mines 1988, 1990; USGS 2006a). Prior to its cessation, U.S. production of arsenic trioxide had been 7,300 metric tons in 1983, 6,800 metric tons in 1984, and 2,200 metric tons in 1985 (U.S. Bureau of Mines 1988). In 2005, arsenic trioxide was obtained from the treatment of nonferrous ores or concentrates in 14 countries. In 2005, the world’s largest producer of arsenic trioxide was China, followed by Chile and Peru. China is the world leader in the production of commercial-grade arsenic followed by Japan. The United States, with an apparent demand of 8,800 metric tons in 2005, is the world's leading consumer of arsenic, mainly for CCA. This is an increase over 2004 with an apparent demand of 6,800 metric tons, but far less than that of 2003, 21,600 metric tons (USGS 2006a).

Tables 5-1 and 5-2 list facilities in each state that manufacture or process arsenic and arsenic compounds, respectively, as well as the intended use and the range of maximum amounts of arsenic or arsenic compounds that are stored on site. In 2004, there were 58 and 361 reporting facilities that produced, processed, or used arsenic and arsenic compounds, respectively, in the United States. The data listed in Tables 5-1 and 5-2 are derived from the Toxics Release Inventory (TRI04 2006). Only certain types of facilities were required to report. Therefore, this is not an exhaustive list. Current U.S. manufacturers of selected arsenic compounds are given in Table 5-3.

Table 5-1. Facilities that Produce, Process, or Use Arsenic

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
AK	1	1,000,000	9,999,999	1, 13
AL	18	0	9,999,999	1, 2, 3, 5, 7, 8, 11, 12, 13, 14
AR	4	1,000	999,999	7, 8
AZ	9	0	99,999	1, 3, 4, 5, 8, 12, 13
CA	31	0	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13
CO	8	0	999,999	2, 7, 8, 11, 12
FL	10	1,000	999,999	1, 3, 5, 7, 8, 11, 12
GA	16	100	49,999,999	2, 3, 4, 6, 7, 8, 11, 12, 13, 14
HI	1	10,000	99,999	8
IA	4	100	99,999	6, 7, 8
ID	7	0	49,999,999	1, 2, 3, 5, 6, 7, 9, 12, 13
IL	16	0	999,999	1, 3, 4, 5, 6, 7, 8, 12, 14
IN	17	0	999,999	1, 3, 5, 6, 7, 8, 9, 12, 13
KY	9	0	999,999	1, 2, 3, 5, 6, 7, 8, 11
LA	8	0	999,999	1, 2, 3, 7, 8, 12, 13
MA	5	1,000	999,999	3, 7, 8
MD	9	0	999,999	1, 2, 4, 5, 6, 7, 8
MI	10	0	999,999	3, 7, 8, 12, 13
MN	5	100	99,999	1, 7, 8, 13
MO	6	100	999,999	1, 2, 3, 4, 5, 6, 7, 8
MS	9	1,000	49,999,999	2, 3, 4, 7, 8, 9
NC	21	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 13, 14
ND	2	0	99,999	8
NE	1	0	99	8
NJ	9	0	99,999	1, 2, 3, 5, 7, 8, 9
NM	2	10,000	999,999	7, 12
NV	6	1,000	99,999,999	1, 2, 4, 5, 6, 7, 8, 11, 12, 13
NY	4	0	99,999	7, 8, 12
OH	15	0	999,999	1, 2, 3, 4, 5, 8, 9, 12, 13
OK	9	0	99,999	1, 2, 5, 6, 7, 9, 11, 12, 13
OR	6	10,000	999,999	1, 5, 7, 8, 12
PA	24	0	999,999	1, 2, 3, 5, 6, 7, 8, 10, 11, 12, 13
PR	3	1,000	99,999	8, 11
SC	9	0	9,999,999	1, 2, 3, 5, 6, 8, 12
SD	1	10,000,000	49,999,999	1, 7, 11, 13
TN	11	0	999,999	1, 2, 3, 6, 7, 8, 11, 12, 14
TX	29	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14
VA	8	0	999,999	2, 3, 7, 8, 10

Table 5-1. Facilities that Produce, Process, or Use Arsenic

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
WA	3	0	99,999	5, 7, 8
WI	9	0	99,999	1, 2, 3, 4, 5, 6, 7, 8, 12
WV	19	100	999,999	1, 2, 3, 5, 7, 8, 10, 11, 12
WY	1	100	999	1, 13

[a]Post office state abbreviations used
[b]Amounts on site reported by facilities in each state
[c]Activities/Uses:

1. Produce
2. Import
3. Onsite use/processing
4. Sale/Distribution
5. Byproduct
6. Impurity
7. Reactant
8. Formulation Component
9. Article Component
10. Repackaging
11. Chemical Processing Aid
12. Manufacturing Aid
13. Ancillary/Other Uses
14. Process Impurity

Source: TRI04 2006 (Data are from 2004)

Table 5-2. Facilities that Produce, Process, or Use Arsenic Compounds

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
AK	6	1,000	49,999,999	1, 5, 7, 12, 13, 14
AL	37	0	499,999,999	1, 2, 3, 4, 5, 7, 8, 9, 11, 12, 13
AR	20	1,000	99,999,999	1, 2, 3, 7, 8, 9, 11, 12, 13, 14
AZ	29	100	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
CA	40	100	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14
CO	9	1,000	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12
DE	1	10,000	99,999	1, 5, 9
FL	30	0	999,999	1, 3, 4, 5, 7, 8, 9, 11, 12, 13, 14
GA	50	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14
HI	6	1,000	99,999	7, 8, 11
IA	22	0	9,999,999	1, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13
ID	6	10,000	9,999,999	1, 3, 5, 6, 7, 8, 9, 12, 13
IL	44	0	999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
IN	54	0	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14
KS	14	0	999,999	1, 3, 4, 5, 6, 7, 8, 12, 13, 14
KY	29	0	999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
LA	32	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13
MA	9	0	999,999	1, 4, 5, 6, 7, 8
MD	19	0	999,999	1, 4, 5, 7, 8, 9, 11, 12, 13
ME	2	1,000	99,999	7
MI	32	0	999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14
MN	13	0	999,999	1, 3, 4, 5, 7, 8, 9, 11, 12, 13
MO	32	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14
MS	28	1,000	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13
MT	8	1,000	10,000,000,000	1, 2, 3, 4, 5, 6, 7, 12, 13, 14
NC	65	0	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
ND	11	1,000	99,999	1, 5, 8, 9, 11, 12, 13, 14
NE	6	1,000	999,999	1, 2, 3, 4, 5, 6, 8, 9, 12, 13
NH	2	1,000	99,999	8, 11
NJ	35	0	999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 13
NM	11	1,000	499,999,999	1, 5, 7, 12, 13
NV	31	1,000	10,000,000,000	1, 2, 3, 5, 6, 7, 9, 11, 12, 13, 14
NY	27	0	9,999,999	1, 2, 3, 4, 5, 7, 8, 9, 12, 13
OH	50	0	999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14
OK	14	100	9,999,999	1, 2, 3, 4, 5, 6, 8, 12, 13, 14
OR	12	100	99,999	1, 2, 3, 7, 8, 12
PA	53	0	999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14
PR	8	1,000	99,999	1, 2, 3, 5, 8, 11
RI	7	100	99,999	7, 8

Table 5-2. Facilities that Produce, Process, or Use Arsenic Compounds

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
SC	34	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14
SD	6	1,000	99,999,999	1, 5, 6, 7, 8, 11, 12, 13
TN	29	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
TX	54	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14
UT	23	0	499,999,999	1, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13
VA	24	0	499,999,999	1, 2, 3, 4, 5, 7, 8, 9, 11, 12, 13, 14
WA	14	0	999,999	1, 2, 3, 4, 5, 7, 8, 9, 11, 12, 13
WI	13	100	99,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 11
WV	26	0	999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 13, 14
WY	9	1,000	99,999	1, 3, 4, 5, 7, 8, 9, 12, 13

[a]Post office state abbreviations used
[b]Amounts on site reported by facilities in each state
[c]Activities/Uses:

1. Produce
2. Import
3. Onsite use/processing
4. Sale/Distribution
5. Byproduct
6. Impurity
7. Reactant
8. Formulation Component
9. Article Component
10. Repackaging
11. Chemical Processing Aid
12. Manufacturing Aid
13. Ancillary/Other Uses
14. Process Impurity

Source: TRI04 2006 (Data are from 2004)

Table 5-3. Current U.S. Manufacturers of Selected Arsenic Compounds[a]

Company	Location(s)
Arsenic acid	
Arch Wood Protection, Inc.	Conley, Georgia
Osmose Wood Preserving, Inc.	Millington, Tennessee
Arsanilic acid	
Fleming Laboratories, Inc.	Charlotte, North Carolina
Copper Chromated Arsenic (CCA)	
Arch Wood Protection, Inc.	Conley, Georgia; Kalama, Washington; Smyrna, Georgia[b]; Valparaiso, Indiana
Chemical Specialties, Inc.[b]	Charlotte, North Carolina
Osmose Wood Preserving, Inc.[b]	Buffalo, New York
Calcium acid methanearsonate (CAMA)	
Drexel Chemical Company (formulator)[c]	No information provided
Disodium methanearsonate (DSMA)	
Drexel Chemical Company	Tunica, Mississippi
Monosodium methyl arsonate (MSMA)	
Drexel Chemical Company	Tunica, Mississippi
Gallium arsenide	
Atomergic Chemetals Corporation	Farmingdale, New York

[a]Derived from Stanford Research Institute (SRI 2006), except where otherwise noted. SRI reports production of chemicals produced in commercial quantities (defined as exceeding 5,000 pounds or $10,000 in value annually) by the companies listed.
[b]USGS 2006a
[c]Meister et al. 2006

5.2 IMPORT/EXPORT

Since U.S. production ceased in 1985, all arsenic consumed in the United States is imported. Imports of arsenic (metal and compounds combined) have increased substantially since the mid-1980s, reaching 8,810 metric tons (as arsenic content) in 2005, of which 812 metric tons was as elemental arsenic. In 2005, 11,000 metric tons of arsenic trioxide was imported into the United States. China is the major import source for elemental arsenic from 2001 to 2004, supplying 81%, followed by Japan (15%) and Hong Kong (2%). China is also the major import source in 2001–2004 for arsenic trioxide, supplying 59% to the United States, followed by Morocco (22%), Chile (7%), and Mexico (5%) (USGS 2006a, 2006b).

U.S. exports of elemental arsenic were 220 metric tons in 2004 and are estimated to be 200 metric tons in 2005 (USGS 2006b). In 2005, U.S. import of arsenic was approximately 8.1×10^5 kilograms (810 metric tons) (ITA 2007a, 2007b).

5.3 USE

In 2003, the United States was the world's largest consumer of arsenic, with an apparent demand of 21,600 metric tons. In 2005, the Unites States was still the world's largest consumer of arsenic, mainly for CCA. Production of wood preservatives, primarily CCA, $CrO_3 \bullet CuO \bullet As_2O_5$, accounted for >90% of domestic consumption of arsenic trioxide prior to 2004. In 2005, about 65% of domestic consumption of arsenic trioxide was used for the production of CCA. The remainder was used for the production of agricultural chemicals, including herbicides, and insecticides. The major U.S. producers of CCA in 2005 included Arch Wood Protection, Inc., Smyrna Georgia; Chemical Specialties Inc., Charlotte, North Carolina; and Osmose Wood Preserving, Inc., Buffalo, New York (USGS 2006a). CCA is the most widely used wood preservative in the world. Wood treated with CCA is referred to as 'pressure treated' wood (American Wood Preservers Association 2007; Page and Loar 1993). In 1997, approximately 727.8 million cubic feet (20.6 million cubic meters) of wood products were pressure treated in the United States. CCA is a water-based product that protects several commercially available species of western lumber from decay and insect attack. It is widely used in treating utility poles, building lumber, and wood foundations. CCA comes in three types, A, B, and C, which contain different proportions of chromium, copper, and arsenic oxides. Type C, the most popular type, contains CrO_3, CuO, and As_2O_5 in the proportions 47.5, 18.5, and 34.0%, respectively. The retention levels are 0.25 pounds per cubic feet (pcf) for above ground use such as fencing and decking, 0.40 pcf for lumber used in ground contact such as

fence posts and deck posts, and 0.60 pcf for all weather wood foundations (Chicago Flameproof 2000; Permapost 2000). Piling used for fresh and saltwater contact should contain 0.80 and 2.5 pcf of CCA, respectively. Ammoniacal copper zinc arsenate (ACZA) is another arsenic containing preservative used to treat wood; however, it is not as widely used as CCA–C (Lebow et al. 2000).

In 2003, U.S. manufacturers of arsenical wood preservatives began a voluntary transition from CCA to other wood preservatives in wood products for certain residential uses, such as play structures, picnic tables, decks, fencing, and boardwalks. This phase out was completed on December 31, 2003; wood treated prior to this date could still be used and structures made with CCA-treated wood would not be affected. CCA-treated wood products continue to be used in industrial applications (EPA 2003a).

Elemental arsenic is used as an alloying element in ammunition and solders, as an anti-friction additive to metals used for bearings, and to strengthen lead-acid storage battery grids. In the past, the predominant use of arsenic was in agriculture. The uses of lead arsenate as a growth regulator on citrus, calcium arsenate as an herbicide on turf, sodium arsenite as a fungicide on grapes, and arsenic acid as a desiccant on okra for seed and cotton were voluntarily cancelled in the late 1980s and the early 1990s (EPA 2006). The herbicides, MSMA and DSMA, are registered for weed control on cotton, for turf grass and lawns, and under trees, vines, and shrubs; calcium acid methanearsonate (CAMA) is registered for postemergent weed control on lawns. Cacodylic acid, a defoliant and herbicide, is registered for weed control under nonbearing citrus trees, around buildings and sidewalks, and for lawn renovation (EPA 2006). Approximately 3 million pounds of MSMA or DSMA, and 100,000 pounds of cacodylic acid are applied in the U.S. annually based on EPA's Screening Level Use Analysis data. Data were not available for CAMA. Application to cotton and turf (residential and golf courses) are the major uses of organic arsenical herbicides. Currently, there are approximately 90, 25, 4, and 35 end-use products containing MSMA, DSMA, CAMA, and cacodylic acid, respectively (EPA 2006).

Other organic arsenicals used in agriculture include arsanilic acid, sodium arsanilate, and 3-nitro-4-hydroxyphenylarsonic acid (roxarsone), which are antimicrobials used in animal and poultry feeds (Beerman 1994). While the U.S. Food and Drug Administration (FDA) has authorized the used of these compounds as medicinal feed additives, only one of the arsenical compounds may be used at a time as the sole source of organic arsenic in the feed (EPA 1998k). In 1999–2000, about 70% of the broiler industry added roxarsone to broiler poultry feed; concentrations of roxarsone in feed range from 22.7 to 45.4 g/ton (Garbarino et al. 2003).

From the mid-nineteenth century to the introduction of organic pesticides in the 1940s, inorganic arsenic compounds were the dominant pesticides available to farmers and fruit growers. Calcium arsenate was formerly used to control the boll weevil and cotton worm and was used as an herbicide. Lead arsenate was used on apple and other fruit orchards as well as on potato fields. Sodium arsenite was used to control weeds on railroad right-of-ways, potato fields, and in industrial areas, as well as in baits and to debark trees. Sodium arsenate had some application in ant traps. The use of inorganic arsenic compounds in agriculture has virtually disappeared beginning around the 1960s (Azcue and Nriagu 1994; Meister 1987; Merwin et al. 1994; Sanok et al. 1995). Food uses were voluntarily cancelled in 1993 as was the use of arsenic acid as a defoliant on cotton plants; inorganic arsenic's remaining allowable uses are in ant baits and wood preservatives (EPA 1999h). In 1987, EPA issued a preliminary decision to cancel the registration of most inorganic arsenicals used as nonwood pesticides (Loebenstein 1994) (see Chapter 8). According to the California Department of Pesticide Regulation, arsenic acid, arsenic pentoxide, and arsenic trioxide are registered currently as pesticides in the United States; there are no active registrants listed for calcium arsenate, lead arsenate, or sodium arsenite (NPIRS 2007).

High-purity arsenic (99.9999%) is used by the electronics industry for gallium-arsenide semiconductors for telecommunications, solar cells, and space research (USGS 2006b). Arsenic trioxide and arsenic acid were used as a decolorizer and fining agent in the production of bottle glass and other glassware (Carapella 1992).

Arsenic compounds have a long history of use in medicine. Inorganic arsenic was used as a therapeutic agent through the mid-twentieth century, primarily for the treatment of leukemia, psoriasis, and chronic bronchial asthma; organic arsenic antibiotics were extensively used in the treatment of spirochetal and protozoal disease (NRC 1999). The availability of inorganic arsenicals in Western medicines ended in the 1970s, although they may still be encountered in non-Western traditional medicines. By the 1980s, the only remaining medicinal organic arsenical was melarsoprol for treatment of the meningoencephalitic stage of African trypanosomiasis. There has been renewed interest in arsenic as a therapeutic agent, namely the use of arsenic trioxide in the treatment of acute promyelocytic leukemia (APL) (Gallagher 1998; Kroemer and de Thé 1999; Miller 1998; Wang 2001). In 2000, the FDA approved arsenic trioxide for this use (FDA 2000).

5.4 DISPOSAL

Wastes containing arsenic are considered hazardous wastes, and as such, their treatment, storage, and disposal are regulated by law (see Chapter 8). The main route of disposal of solid wastes containing arsenic is landfilling. EPA has promulgated rules and treatment standards for landfilling liquid arsenical wastes (EPA 1990e). Arsenic-containing electronic components such as relays, switches, and circuit boards are disposed of at hazardous waste sites, and the elemental arsenic is not reclaimed. Process water at wood treatment plants that contained arsenic contained was reused. Gallium-arsenide scrap from the manufacture of semiconductor devices was reprocessed for arsenic recovery. Arsenic was not recovered from arsenical residues and dusts at domestic nonferrous smelters (USGS 2006b).

CCA-treated wood is classified as nonhazardous waste under the Federal Resource Conservation and Recovery Act (RCRA). CCA-treated wood is disposed of with regular municipal trash (i.e., municipal solid waste, not yard waste). It should not be burned in open fires, stoves, residential boilers, or fire places and should not be composted or used as mulch. Treated wood from commercial or industrial applications may only be burned in commercial or industrial incinerators in accordance with state and federal regulations (Adobe Lumber 2002; EPA 2005a).

Arsenic is listed as a toxic substance under Section 313 of the Emergency Planning and Community Right to Know Act (EPCRA) under Title III of the Superfund Amendments and Reauthorization Act (SARA) (EPA 1995c). Disposal of wastes containing arsenic is controlled by a number of federal regulations (see Chapter 8).

6. POTENTIAL FOR HUMAN EXPOSURE

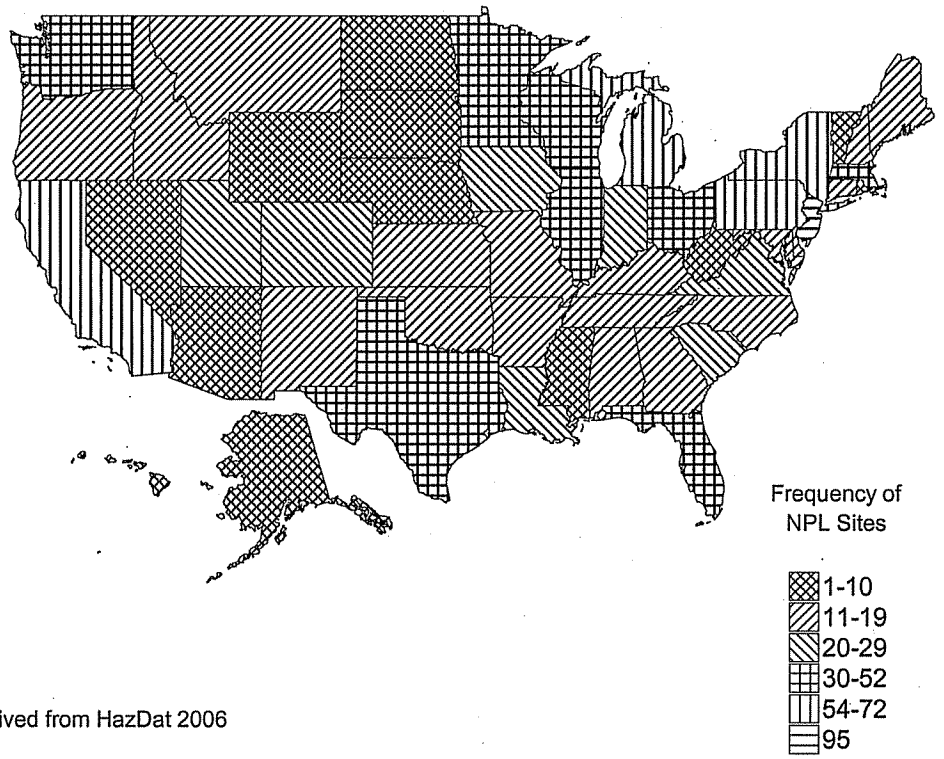
6.1 OVERVIEW

Arsenic has been identified in at least 1,149 of the 1,684 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (HazDat 2006). However, the number of sites evaluated for arsenic is not known. The frequency of these sites can be seen in Figure 6-1. Of these sites, 1,134 are located within the United States and 11, 2, and 2 are located in the Commonwealth of Puerto Rico, the Virgin Islands, and Guam (not shown).

Arsenic is widely distributed in the Earth's crust, which contains about 3.4 ppm arsenic (Wedepohl 1991). It is mostly found in nature in minerals, such as realgar (As_4S_4), orpiment (As_2S_3), and arsenolite (As_2O_3), and only found in its elemental form to a small extent. There are over 150 arsenic-bearing minerals (Budavari et al. 2001; Carapella 1992). While arsenic is released to the environment from natural sources such as wind-blown soil and volcanoes, releases from anthropogenic sources far exceed those from natural sources. Anthropogenic sources of arsenic include nonferrous metal mining and smelting, pesticide application, coal combustion, wood combustion, and waste incineration. Most anthropogenic releases of arsenic are to land or soil, primarily in the form of pesticides or solid wastes. However, substantial amounts are also released to air and water.

Arsenic found in soil either naturally occurring or from anthropogenic releases forms insoluble complexes with iron, aluminum, and magnesium oxides found in soil surfaces, and in this form, arsenic is relatively immobile. However, under reducing conditions, arsenic can be released from the solid phase, resulting in soluble mobile forms of arsenic, which may potentially leach into groundwater or result in runoff of arsenic into surface waters. In aquatic systems, inorganic arsenic occurs primarily in two oxidation states, As(V) and As(III). Both forms generally co-exist, although As(V) predominates under oxidizing conditions and As(III) predominates under reducing conditions. Arsenic may undergo a variety of reactions in the environment, including oxidation-reduction reactions, ligand exchange, precipitation, and biotransformation (EPA 1979, 1984a; Pongratz 1998; Welch et al. 1988). These reactions are influenced by Eh (the oxidation-reduction potential), pH, metal sulfide and sulfide ion concentrations, iron concentration, temperature, salinity, and distribution and composition of the biota (EPA 1979; Wakao et al. 1988). Much of the arsenic will adsorb to particulate matter and sediment. Arsenic released to air exists mainly in the form of particulate matter. Arsenic released from combustion processes will

Figure 6-1. Frequency of NPL Sites with Arsenic Contamination



Derived from HazDat 2006

generally occur as highly soluble oxides. These particles are dispersed by the wind and returned to the earth in wet or dry deposition. Arsines that are released to the atmosphere as a result of microbial action are oxidized to nonvolatile species that settle back to the ground.

Because arsenic is a natural component of the Earth's crust, low levels of the element are found in all environmental media. Atmospheric levels of arsenic in remote locations (away from human releases) range from 1 to 3 ng/m^3, while concentrations in urban areas may range from 20 to 100 ng/m^3. Concentrations in water are usually <10 μg/L, although higher levels may occur near natural mineral deposits or anthropogenic sources. Natural levels of arsenic in soil usually range from 1 to 40 mg/kg, with a mean of 5 mg/kg, although much higher levels may occur in mining areas, at waste sites, near high geological deposits of arsenic-rich minerals, or from pesticide application. Arsenic is also found in many foods, at concentrations that usually range from 20 to 140 μg/kg. Total arsenic concentrations may be substantially higher in certain seafoods. However, the general consensus in the literature is that about 85–>90% of the arsenic in the edible parts of marine fish and shellfish is organic arsenic (e.g., arsenobetaine, arsenochloline, dimethylarsinic acid) and that approximately 10% is inorganic arsenic (EPA 2003b). Drinking water in the United States generally contains an average of 2 μg/L of arsenic (EPA 1982c), although 12% of water supplies from surface water sources in the north Central region of the United States and 12% of supplies from groundwater sources in the western region have levels exceeding 20 μg/L (Karagas et al. 1998). In January 2001, EPA adopted a new standard that arsenic levels in drinking water were not to exceed 10 μg/L, replacing the previous standard of 50 μg/L. The date for compliance with the new MCL was January 23, 2006 (EPA 2001).

For most people, diet is the largest source of exposure to arsenic. Mean dietary intakes of total arsenic of 50.6 μg/day (range of 1.01–1,081 μg/day) and 58.5 μg/day (range of 0.21–1,276 μg/day) has been reported for females and males (MacIntosh et al. 1997). U.S. dietary intake of inorganic arsenic has been estimated to range from 1 to 20 μg/day, with grains and produce expected to be significant contributors to dietary inorganic arsenic intake (Schoof et al. 1999a, 1999b). The predominant dietary source of arsenic is generally seafood. Inorganic arsenic in seafood sampled in a market basket survey of inorganic arsenic in food ranged from <0.001 to 0.002 μg/g (Schoof et al. 1999a, 1999b). Intake of arsenic from air and soil are usually much smaller than that from food and water (Meacher et al. 2002).

People who produce or use arsenic compounds in occupations such as nonferrous metal smelting, pesticide manufacturing or application, wood preservation, semiconductor manufacturing, or glass production may be exposed to substantially higher levels of arsenic, mainly from dusts or aerosols in air.

Exposure at waste sites may occur by a variety of pathways, including inhalation of dusts in air, ingestion of contaminated soil or water, or through the food chain. The magnitude of the exposures can only be evaluated on a site-by-site basis; however, exposures generally do not exceed background intakes from food and drinking water.

Tables 4-1, 4-2, 4-3, and 4-4 summarize all of the names, abbreviations, and structures of the various arsenic compounds that are discussed in Chapter 6.

6.2 RELEASES TO THE ENVIRONMENT

The Toxics Release Inventory (TRI) data should be used with caution because only certain types of facilities are required to report (EPA 2005k). This is not an exhaustive list. Manufacturing and processing facilities are required to report information to the TRI only if they employ 10 or more full-time employees; if their facility is included in Standard Industrial Classification (SIC) Codes 10 (except 1011, 1081, and 1094), 12 (except 1241), 20–39, 4911 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4931 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4939 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4953 (limited to facilities regulated under RCRA Subtitle C, 42 U.S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited to facilities primarily engaged in solvents recovery services on a contract or fee basis); and if their facility produces, imports, or processes ≥25,000 pounds of any TRI chemical or otherwise uses >10,000 pounds of a TRI chemical in a calendar year (EPA 2005k).

6.2.1 Air

Estimated releases of 4,800 pounds (~2.2 metric tons) of arsenic to the atmosphere from 58 domestic manufacturing and processing facilities in 2004, accounted for about 0.52% of the estimated total environmental releases from facilities required to report to the TRI (TRI04 2006). Estimated releases of 0.13 million pounds (~59 metric tons) of arsenic compounds to the atmosphere from 361 domestic manufacturing and processing facilities in 2004, accounted for about 0.11% of the estimated total environmental releases from facilities required to report to the TRI (TRI04 2006). These releases for arsenic and arsenic compounds are summarized in Table 6-1 and 6-2, respectively.

Table 6-1. Releases to the Environment from Facilities that Produce, Process, or Use Arsenic[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
AL	1	51	162	0	110,264	0	110,425	52	110,477
AR	2	0	0	No data	0	0	No data	0	0
AZ	2	10	0	0	20,717	0	20,727	0	20,727
CA	3	13	14	0	5,482	0	13	5,497	5,510
FL	2	4	0	0	0	4,950	4	4,950	4,954
GA	4	8	10	0	1,603	5	13	1,613	1,626
IA	1	0	1	0	0	0	0	1	1
ID	1	39	0	0	361,252	0	361,291	0	361,291
IL	2	250	129	0	14,087	0	379	14,087	14,466
IN	1	5	5	0	13,250	250	5	13,505	13,510
KS	1	0	0	No data	0	0	No data	0	0
KY	1	0	1	0	0	6	1	6	7
MI	2	0	5	0	0	750	5	750	755
MN	1	15	47	0	14,504	0	15	14,551	14,566
MO	1	5	0	0	0	4,040	5	4,040	4,045
MS	2	0	0	0	0	0	0	0	0
NC	4	35	8	0	1	1	43	2	45
NV	1	0	0	0	0	0	0	0	0
NY	4	0	1	0	26,525	1	26,401	126	26,527
OH	2	13	0	0	0	0	13	0	13
OR	1	0	0	0	92,606	0	92,606	0	92,606
PA	5	166	8	0	14,362	26,140	199	40,477	40,676
SC	3	10	10	0	0	1,002	15	1,007	1,022
TN	3	3,988	0	0	0	0	3,988	0	3,988
TX	5	139	376	168,563	12,600	0	181,636	42	181,678

Table 6-1. Releases to the Environment from Facilities that Produce, Process, or Use Arsenic[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
WI	2	15	0	0	760	0	15	760	776
WV	1	0	0	0	10,135	0	10,135	0	10,135
Total	58	4,766	778	168,563	698,149	37,145	807,935	101,466	909,401

[a]The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list. Data are rounded to nearest whole number.
[b]Data in TRI are maximum amounts released by each facility.
[c]Post office state abbreviations are used.
[d]Number of reporting facilities.
[e]The sum of fugitive and point source releases are included in releases to air by a given facility.
[f]Surface water discharges, waste water treatment-(metals only), and publicly owned treatment works (POTWs) (metal and metal compounds).
[g]Class I wells, Class II-V wells, and underground injection.
[h]Resource Conservation and Recovery Act (RCRA) subtitle C landfills; other on-site landfills, land treatment, surface impoundments, other land disposal, other landfills.
[i]Storage only, solidification/stabilization (metals only), other off-site management, transfers to waste broker for disposal, unknown
[j]The sum of all releases of the chemical to air, land, water, and underground injection wells.
[k]Total amount of chemical transferred off-site, including to POTWs.

RF = reporting facilities; UI = underground injection

Source: TRI04 2006 (Data are from 2004)

Table 6-2. Releases to the Environment from Facilities that Produce, Process, or Use Arsenic Compounds[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
AK	1	511	0	1,400,000	1,200,000	0	2,600,511	0	2,600,511
AL	19	4,299	18,127	0	853,469	7,555	875,876	7,574	883,450
AR	12	0	0	0	133	26,435	0	26,568	26,568
AZ	5	5,421	0	0	402,335	422	394,749	13,429	408,178
CA	5	65	14	0	355,660	86,396	160,673	281,461	442,134
CO	1	11	0	0	4,094	0	4,105	0	4,105
CT	1	0	0	0	0	0	No data	0	0
FL	15	3,208	503	0	343,508	4,057	346,310	4,966	351,276
GA	23	8,643	7,823	0	422,124	5,127	437,496	6,221	443,717
HI	1	0	0	0	0	0	No data	0	0
IA	4	1,291	482	0	0	35,324	1,773	35,324	37,097
ID	3	332	20	0	1,056,904	0	1,057,256	0	1,057,256
IL	11	3,960	3,110	0	96,093	21,038	71,819	52,382	124,202
IN	21	13,786	8,282	0	768,297	42,808	632,704	200,470	833,174
KS	4	924	0	0	12,082	1	13,006	1	13,007
KY	18	14,406	8,427	0	616,074	95,285	578,080	156,112	734,192
LA	7	265	23	0	25,426	0	25,563	151	25,714
MA	1	0	0	0	0	500	0	500	500
MD	8	1,870	291	0	34,130	114,115	2,661	147,745	150,406
MI	10	1,123	2,310	68,924	101,857	1,059	77,505	97,769	175,274
MN	2	10	130	0	19,270	0	19,410	0	19,410
MO	6	462	116	0	27,855	936	10,026	19,343	29,369
MS	6	61	121	0	11,676	46	11,228	676	11,904
MT	3	630	0	0	2,138,190	37	2,138,820	37	2,138,857
NC	15	5,626	4,732	0	168,030	2,429	178,388	2,429	180,818
ND	6	6,326	5	0	318,175	0	137,961	186,545	324,506
NE	2	180	0	0	11,000	0	11,180	0	11,180
NJ	2	0	1	0	0	8	0	9	9
NM	2	130	0	0	18,326	0	18,456	0	18,456
NV	10	3,041	30,017	0	98,894,564	0	98,927,328	294	98,927,622
NY	3	67	36	0	27,059	802	27,141	823	27,964
OH	17	8,595	8,352	81,024	741,730	274	668,157	171,818	839,975
OK	4	115	13	0	25,000	4,202	115	29,215	29,330
OR	4	0	5	0	0	4,012	5	4,012	4,017
PA	23	18,963	2,166	0	666,753	69,053	403,582	353,353	756,935
PR	3	0	0	0	0	0	No data	0	0
RI	1	0	8	0	0	1,006	8	1,006	1,014

Table 6-2. Releases to the Environment from Facilities that Produce, Process, or Use Arsenic Compounds[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
SC	13	2,178	1,443	0	25,817	22,705	29,438	22,705	52,143
SD	1	0	0	0	0	0	No data	0	0
TN	13	3,379	25,878	0	292,914	17,219	258,643	80,746	339,389
TX	17	4,616	199	33,148	196,385	31,557	226,751	39,155	265,906
UT	5	6,715	4,500	0	6,368,500	3,500	6,379,715	3,500	6,383,215
VA	11	1,911	2,773	0	160,154	8,463	164,789	8,512	173,301
WA	4	0	0	0	0	0	No data	0	0
WI	4	94	21	0	1,313	9,216	223	10,421	10,644
WV	12	2,693	2,417	0	536,628	10,000	441,237	110,501	551,738
WY	2	3,300	0	0	10,800	0	14,100	0	14,100
Total	361	129,205	132,347	1,583,096	116,952,326	625,588	117,346,787	2,075,775	119,422,562

[a]The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list. Data are rounded to nearest whole number.
[b]Data in TRI are maximum amounts released by each facility.
[c]Post office state abbreviations are used.
[d]Number of reporting facilities.
[e]The sum of fugitive and point source releases are included in releases to air by a given facility.
[f]Surface water discharges, waste water treatment-(metals only), and publicly owned treatment works (POTWs) (metal and metal compounds).
[g]Class I wells, Class II-V wells, and underground injection.
[h]Resource Conservation and Recovery Act (RCRA) subtitle C landfills; other on-site landfills, land treatment, surface impoundments, other land disposal, other landfills.
[i]Storage only, solidification/stabilization (metals only), other off-site management, transfers to waste broker for disposal, unknown
[j]The sum of all releases of the chemical to air, land, water, and underground injection wells.
[k]Total amount of chemical transferred off-site, including to POTWs.

RF = reporting facilities; UI = underground injection

Source: TRI04 2006 (Data are from 2004)

Arsenic naturally occurs in soil and will be present in the atmosphere as airborne dust. It is also emitted from volcanoes and in areas of dormant volcanism (e.g., fumaroles). Gaseous alkyl arsenic compounds may be released from soil that has been treated with inorganic arsenic compounds as a result of biogenic processes (Schroeder et al. 1987; Tamaki and Frankenberger 1992). Arsenic naturally occurs in sea water and vegetation and is released into the atmosphere in sea salt spray and forest fires. Anthropogenic sources of arsenic include nonferrous metal smelting, coal, oil and wood combustion, and municipal waste incineration. Arsenic naturally occurs in coal and oil and therefore, coal- and oil-fired power plants release arsenic to the atmosphere in their emissions (Pacyna 1987). Arsenic's use in agriculture and industrial processes also contributes to its emissions. One important source of arsenic emissions is cotton ginning in which the cotton seeds are removed from the raw cotton.

The National Air Toxics Assessment reported that total anthropogenic emissions for arsenic compounds in the United States in 1996 were 355 tons/year (EPA 2005b). EPA conducted a modeling study with the Assessment System for Population Exposure Nationwide (ASPEN) in which estimates of emissions of hazardous air pollutants were used to estimate air quality (Rosenbaum et al. 1999). Using 1990 data, the total emissions of arsenic in the conterminous 48 states, excluding road dust or windblown dust from construction or agricultural tilling was estimated to be 3.0 tons/day with 90% of emissions coming from point sources and 5% each from area and mobile sources. U.S. emissions of arsenic to the atmosphere were estimated as 3,300 metric tons per year between 1979 and 1986 (Pacyna et al. 1995). There is evidence that anthropogenic emissions, at least from smelters, are lower than they had been in the early 1980s. It is likely that air releases of arsenic decreased during the 1980s due to regulations on industrial emissions (EPA 1986f), improved control technology for coal-burning facilities, and decreased use of arsenical pesticides.

Nriagu and Pacyna (1988) and Pacyna et al. (1995) estimated worldwide emissions of arsenic to the atmosphere for 1983. Estimates of yearly emissions from anthropogenic sources ranged from 12,000 to 25,600 metric tons with a median value of 18,800 metric tons. Natural sources contributed 1,100–23,500 metric tons annually. Chilvers and Peterson (1987) estimated global natural and anthropogenic arsenic emissions to the atmosphere as 73,500 and 28,100 metric tons per year, respectively. Copper smelting and coal combustion accounted for 65% of anthropogenic emissions. A U.S. Bureau of Mines study on the flow of mineral commodities estimated that global emissions of arsenic from metal smelting, coal burning, and other industrial uses ranged from 24,000 to 124,000 metric tons per year compared to natural releases, mostly from volcanoes, ranging from 2,800 to 8,000 metric tons per year (Loebenstein 1994).

Pirrone and Keeler (1996) compared trends of trace element emissions from major anthropogenic sources in the Great Lakes region with ambient concentrations observed in urban areas of the region. They found that arsenic emissions increased about 2.8% per year from 1982 to 1988 and then decreased steadily by about 1.4% per year to 1993. Coal combustion in electric utilities and in residential, commercial, and industrial facilities was an important source of arsenic in the region, accounting for about 69% of the total emissions. Iron-steel manufacturing accounted for about 13% of the region wide arsenic emissions and nonferrous metals production for 17%.

Arsenic in the particulate phase is the predominant (89–98.6%) form of arsenic in the troposphere (Matschullat 2000). Inorganic species, most commonly trivalent arsenic, is the dominant form of arsenic in the air over emission areas; methylated forms of arsenic are probably of minor significance. Arsenic-containing air samples of smelter or coal-fired power plant origin consist largely of trivalent arsenic in both vapor and particulate form (Pacyna 1987). Oxides are the primary species evolved from fossil fuel and industrial processes. Additionally, arsenic trisulfide has also been reported from coal combustion, organic arsines from oil combustion, and arsenic trichloride from refuse incineration.

Arsenic has been identified in 59 air samples collected from 1,684 current or former NPL hazardous waste sites where it was detected in some environmental media (HazDat 2006).

6.2.2 Water

Estimated releases of 780 pounds (~0.35 metric tons) of arsenic to surface water from 58 domestic manufacturing and processing facilities in 2004, accounted for about 0.09% of the estimated total environmental releases from facilities required to report to the TRI (TRI04 2006). Estimated releases of 1.3x105 pounds (~59 metric tons) of arsenic compounds to surface water from 361 domestic manufacturing and processing facilities in 2004, accounted for about 0.11% of the estimated total environmental releases from facilities required to report to the TRI (TRI04 2006). These releases for arsenic and arsenic compounds are summarized in Tables 6-1 and 6-2, respectively.

Arsenic may be released to water from the natural weathering of soil and rocks, and in areas of vulcanism. Arsenic may also leach from soil and minerals into groundwater. Anthropogenic sources of arsenic releases to water include mining, nonferrous metals, especially copper, smelting, waste water, dumping of sewage sludge, coal burning power plants, manufacturing processes, urban runoff, atmospheric deposition and poultry farms (Garbarino et al. 2003; Nriagu and Pacyna 1988; Pacyna et al. 1995). A contributory

part of mining and coal burning power plants is leaching from abandoned mine tailing and fly ash waste piles. Significant amounts of arsenic are released in liquid effluents from gold-milling operations using cyanide (Environment Canada 1993). Nriagu and Pacyna (1988) and Pacyna et al. (1995) estimated global anthropogenic inputs of arsenic into rivers, lakes, and oceans for 1983; annual estimated inputs ranged from 11,600 to 70,300 metric tons with a median value of 41,800 metric tons. Arsenic was detected in 58% of samples of urban storm water runoff from 8 of 15 cities surveyed in the National Urban Runoff Program at concentrations ranging from 1 to 50.5 μg/L (Cole et al. 1984).

Leaching of arsenic from soil, landfills, or slag deposits is a source of arsenic in groundwater (Francis and White 1987; Wadge and Hutton 1987). The arsenic in soil may be naturally-occurring or a result of the application of arsenic-containing pesticides or sludge. Wood treated with CCA is used in piers, piling and bulkheads and arsenic can leach from the treated wood (Breslin and Adler-Ivanbrook 1998; Brooks 1996; Cooper 1991; Sanders et al. 1994; Weis et al. 1998). Ammoniacal copper zinc arsenate (ACZA) is another arsenic-containing waterborne preservative; however, it is not as widely used as CCA (Lebow et al. 2000).

Arsenic has been identified in 846 groundwater and 414 surface water samples collected from 1,684 NPL hazardous waste sites, where it was detected in some environmental media (HazDat 2006).

6.2.3 Soil

Estimated releases of 0.70 million pounds (~320 metric tons) of arsenic to soils from 58 domestic manufacturing and processing facilities in 2004, accounted for about 77% of the estimated total environmental releases from facilities required to report to the TRI (TRI04 2006). An additional 0.17 million pounds (~77 metric tons), constituting about 19% of the total environmental emissions, were released via underground injection (TRI04 2006). Estimated releases of 117 million pounds (~5.3x104 metric tons) of arsenic compounds to soils from 361 domestic manufacturing and processing facilities in 2004, accounted for about 98% of the estimated total environmental releases from facilities required to report to the TRI (TRI04 2006). An additional 1.6 million pounds (~720 metric tons), constituting about 1.3% of the total environmental emissions, were released via underground injection (TRI04 2006). These releases for arsenic and arsenic compounds are summarized in Tables 6-1 and 6-2, respectively.

The soil receives arsenic from a variety of anthropogenic sources, including ash residue from power plants, smelting operations, mining wastes, and municipal, commercial, and industrial waste. Ash from

power plants is often incorporated into cement and other materials that are used for roads and construction. Arsenic may be released from such material into soil. Nriagu and Pacyna (1988) and Pacyna et al. (1995) estimated global anthropogenic inputs of arsenic into soil for 1983. Excluding mine tailings and smelter slag, annual estimated inputs ranged from 52,000 to 112,000 metric tons with a median value of 82,000 metric tons. Mine tailings and smelter slag were estimated to add an additional 7,200–11,000 and 4,500–9,000 metric tons, respectively. Old abandoned mine tailings undoubtedly contribute still more. Wood treated with CCA used in foundations or posts could potentially release arsenic into the surrounding soil. CCA preservatives have been shown to leach to varying degrees from wood, as well as through soils in both field and laboratory studies (Chirenje et al. 2003a; Hingston et al. 2001; Lebow et al. 2000; Rahman et al. 2004; Stilwell and Graetz 2001; USDA/USDT 2000). Arsenic may also be released on land through the application of pesticides and fertilizer. Senesi et al. (1999) reported the range of arsenic in 32 fertilizers as 2.2–322 ng/g. Roxarsone (3-nitro-4-hydroxyphenyl-arsonic acid), which was used to treat poultry feed in approximately 70% of the broiler poultry operations in 1999–2000, is excreted unchanged in the manure. Poultry litter (manure and bedding) is routinely used as fertilizer to cropland and pasture. In 2000, assuming 70% of the 8.3 billion broiler poultry produced in the United States were fed roxarsone-treated feed, the resulting manure would contain approximately 2.5×10^5 kg of arsenic (Garbarino et al. 2003). Land application of sewage sludge is another source of arsenic in soil. Arsenic was detected in sewage sludge samples from 23 cities at concentrations of 0.3–53 μg/g (Mumma et al. 1984).

Arsenic has been identified in 758 soil and 515 sediment samples collected from 1,684 NPL hazardous waste sites, where it was detected in some environmental media (HazDat 2006).

6.3 ENVIRONMENTAL FATE

6.3.1 Transport and Partitioning

Arsenic in soil may be transported by wind or in runoff or may leach into the subsurface soil. However, because many arsenic compounds tend to partition to soil or sediment under oxidizing conditions, leaching usually does not transport arsenic to any great depth (EPA 1982c; Moore et al. 1988; Pantsar-Kallio and Manninen 1997; Welch et al. 1988). Arsenic is largely immobile in agricultural soils; therefore, it tends to concentrate and remain in upper soil layers indefinitely. Downward migration has been shown to be greater in a sandy soil than in a clay loam (Sanok et al. 1995). Arsenic from lead arsenate that was used for pest control did not migrate downward below 20 cm in one fruit orchard; in another orchard, 15 years after sludge amendments and deep plowing, essentially all arsenic residues

remained in the upper 40 cm of soil (Merwin et al. 1994). Leaching of arsenic in polluted wetland soil was low; leaching was correlated with the amount of dissolved organic matter in the soil (Kalbitz and Wennrich 1998). The effect of soil characteristics, namely pH, organic matter content, clay content, iron oxide content, aluminum oxide content, and cation exchange capacity (CEC), on the adsorption of various metals, including the metalloid arsenic, to 20 Dutch surface soils was assessed by regression analysis (Janssen et al. 1997). The most influential parameter affecting arsenic adsorption was the iron content of the soil.

Arsenic that is adsorbed to iron and manganese oxides may be released under reducing conditions, which may occur in sediment or flooding conditions (LaForce et al. 1998; McGeehan 1996; Mok and Wai 1994). In addition to reductive dissolution, when nutrient levels are adequate, microbial action can also result in dissolution (LaForce et al. 1998). Interestingly, drying of the previously flooded soil increases arsenic adsorption, possibly due to alterations in iron mineralogy (McGeehan et al. 1998).

Darland and Inskeep (1997) conducted a study to determine the effects of pH and phosphate competition on the transport of arsenate ($H_xAsO_4^{x-3}$) through saturated columns filled with sand containing free iron oxides. At pH 4.5 and 6.5, arsenate transport was strongly retarded, while at pH 8.5, it was rapid. The enhanced transport of arsenate at pH 8 is consistent with the pH dependence of surface complexation reactions describing arsenate sorption by metal oxide minerals that can be categorized as a ligand exchange mechanism. Phosphate was shown to compete effectively with arsenate for adsorption sites on the sand, but the competition was not sufficient to desorb all of the arsenate in batch column experiments, even when the applied phosphate exceeded the column adsorption capacity by a factor of two. The researchers concluded that arsenate desorption kinetics may play an important role in the transport of arsenate through porous media. In a study looking at the effect of competing anions on the adsorption of arsenite and arsenate on ferrihydrite, the effect of phosphate on arsenate adsorption was greater at higher pH than at low pH and the opposite trend was observed for arsenite. While sulfate did not change the affinity of arsenate for ferrihydrite, sulfate reduced the adsorption of arsenite at pHs below 7.0 (Jain and Loeppert 2000).

Smith et al. (1999) investigated the sorption properties of both As(V) and As(III) in 10 Australian soils of widely different chemistry and mineralogy at commonly found arsenic levels. Adsorption of both arsenate and arsenite was rapid (1 hour). The amount of As(V) sorbed varied widely (1.7–62.0 L/kg); soils with lower amounts of oxidic material adsorbed much less arsenic than those with higher amounts of these minerals. Arsenate sorption was highly correlated with the iron oxide content of the soil and this

factor probably accounts for much of the variation in soil adsorptivity. Considerable leaching of arsenic occurred at a separate site where cattle were treated with a dip containing arsenic (cattle dip site) and that contained similar soil properties to that studied by Smith et al. (1999). Arsenite adsorption, which was investigated in four of the Australian soils, was sorbed to a lesser extent than was arsenate. This was attributed to soil minerology and the species of As(V) (arsenate) and As(III) (arsenite) present in solution; at pH 5–7, the dominant As(V) species are $H_2AsO_4^-$ and $HAsO_4^{2-}$ and neutral H_3AsO_3 is the dominant As(III) species. For soils containing low amounts of oxidic minerals, pH had little effect on As(V) sorption, while for oxidic soils, a decrease in sorption was evident as the pH increased. In contrast, As(III) sorption increased with increasing pH (Smith et al. 1999). Jain et al. (1999) reported similar results where arsenite were both found to bind strongly to iron oxides; however, the adsorption of arsenate decreases with increasing pH, while the adsorption of arsenite increases with increasing pH (Jain et al. 1999). As(III), which exists in a neutral form as arsenous acid, H_3AsO_3 (pK_a=9.23, 12.13, 13.4), is less strongly adsorbed on mineral surfaces than the oxyanions of arsenic acid, H_3AsO_4, (pK_a=2.22, 6.98, 11.53) (NRC 1999). Based on its pK_a values, arsenic acid would exist as a mixture of arsenate anions, $H_2AsO_4^-$ and $HAsO_4^{2-}$, under most environmental conditions (pH 5–9).

The practice of liming to remediate contaminated soils and mine tailings has the potential to mobilize arsenic. Experiments performed by Jones et al. (1997) indicate that the increased mobility appears to be consistent with the pH dependence of sorption reactions of arsenic on iron oxide minerals rather than dissolution-precipitation reactions involving arsenic. They recommend that remediation of acidic mine tailings or other arsenic-contaminated soils be carefully evaluated with respect to potential arsenic mobilization, especially at contaminated sites hydraulically connected to surface or groundwaters.

Transport and partitioning of arsenic in water depends upon the chemical form (oxidation state and counter ion) of the arsenic and on interactions with other materials present. Soluble forms move with the water, and may be carried long distances through rivers (EPA 1979). However, arsenic may be adsorbed from water onto sediments or soils, especially clays, iron oxides, aluminum hydroxides, manganese compounds, and organic material (EPA 1979, 1982c; Welch et al. 1988). Under oxidizing and mildly reducing conditions, groundwater arsenic concentrations are usually controlled by adsorption rather than by mineral precipitation. The extent of arsenic adsorption under equilibrium conditions is characterized by the distribution coefficient, K_d, which measures the equilibrium partitioning ratio of adsorbed to dissolved contaminant. The value of K_d depends strongly upon the pH of the water, the arsenic oxidation state, and the temperature. In acidic and neutral waters, As(V) is extensively adsorbed, while As(III) is relatively weakly adsorbed. Trivalent inorganic arsenic exists predominantly as arsenous acid (H_3AsO_3)

at environmental pH and is not strongly adsorbed to suspended solids and sediments in the water column. Pentavalent inorganic arsenic exists predominantly as $H_2AsO_4^-$ and $HAsO_4^{2-}$ in most environmental waters, which has considerably greater adsorption characteristics than arsenous acid. While in acidic and neutral waters, As(V) is more strongly adsorbed relative to As(III), in high-pH waters (pH >9) aquifer K_d values are considerably lower for both oxidation states (Mariner et al. 1996). Sediment-bound arsenic may be released back into the water by chemical or biological interconversions of arsenic species (see Section 6.3.2).

Arsenic enters rivers from where mining operations occurred and is transported downstream, moving from water and sediment into biofilm (attached algae, bacterial, and associated fine detrital material), and then into invertebrates and fish. The source of arsenic in the water column may be resuspended sediment. While arsenic bioaccumulates in animals, it does not appear to biomagnify between tropic levels (Eisler 1994; Farag et al. 1998; Williams et al. 2006).

Most anthropogenic arsenic emitted to the atmosphere arises from high temperature processes (e.g., coal and oil combustion, smelting operations, and refuse incineration) and occurs as fine particles with a mass median diameter of about 1 μm (Coles et al. 1979; Pacyna 1987). These particles are transported by wind and air currents until they are returned to earth by wet or dry deposition. Their residence time in the atmosphere is about 7–9 days, in which time the particles may be transported thousands of kilometers (EPA 1982b; Pacyna 1987). Long-range transport was evident in analyzing deposition of arsenic in countries like Norway; there was no indication that the marine environment contributed significantly to the deposition (Steinnes et al. 1992). Atmospheric fallout can be a significant source of arsenic in coastal and inland waters near industrial areas. Scudlark et al. (1994) determined the average wet depositional flux of arsenic as 49 μg As/m^2/year for two sites in Chesapeake Bay, Maryland from June 1990 to July 1991. They found a high degree of spatial and temporal variability. The elemental fluxes derived predominantly from anthropogenic sources. Golomb et al. (1997) report average total (wet + dry) deposition rates to Massachusetts Bay of 132 μg/m^2/year, of which 21 μg/m^2/year was wet deposition during the period September 15, 1992–September 16, 1993. Hoff et al. (1996) estimated the following arsenic loadings into the Great Lakes for 1994 (lake, wet deposition, dry deposition): Superior, 11,000 kg/year, 3,600 kg/year; Michigan, 5,000 kg/year, 1,800 kg/year; Erie, 5,500 kg/year, 1,800 kg/year; and Ontario, 3,000 kg/year, 580 kg/year. The measured dry deposition fluxes of arsenic at four sampling sites around Lake Michigan ranged approximately from 0.01 to 1.5 μg As/m^2/day; estimated inputs of arsenic into Lake Michigan were reported to be 1.4x10^3 kg/year (Shahin et al. 2000).

Terrestrial plants may accumulate arsenic by root uptake from the soil or by absorption of airborne arsenic deposited on the leaves, and certain species may accumulate substantial levels (EPA 1982b). Yet, even when grown on highly polluted soil or soil naturally high in arsenic, the arsenic level taken up by the plants is comparatively low (Gebel et al. 1998b; Pitten et al. 1999). Kale, lettuce, carrots, and potatoes were grown in experimental plots surrounding a wood preservation factory in Denmark where waste wood was incinerated to investigate the amount and pathways for arsenic uptake by plants (Larsen et al. 1992). On incineration, the arsenate in the wood preservative was partially converted to arsenite; the arsenic emitted from the stack was primarily particle bound. Elevated levels of inorganic arsenic were found in the test plants and in the soil around the factory. Statistical analyses revealed that the dominating pathway for transport of arsenic from the factory to the leafy vegetables (kale) was by direct atmospheric deposition, while arsenic in the root crops (potatoes and carrots) was a result of both soil uptake and atmospheric deposition. Arsenic accumulation by plants is affected by arsenic speciation. Uptake of four arsenic species (arsenite, arsenate, methylarsonic acid, and dimethylarsinic acid) by turnips grown under soilless culture conditions showed that while uptake increased with increasing arsenic concentration in the nutrient, the organic arsenicals showed higher upward translocation than the inorganic arsenical (Carbonell-Barrachina et al. 1999). The total amount of arsenic taken up by the turnip plants (roots and shoots) followed the trend methylarsenate (MMA)<dimethylarsinic acid (DMA) <arsenite<arsenate. In a similar experiment, conducted with tomato plants, the total amount of arsenic taken up by the tomato plants followed the trend DMA<MMA<arsenate≈arsenite, with arsenic concentrations in the plants increasing with increasing arsenic concentration in the nutrient solution. Arsenic was mainly accumulated in the root system (85%) with smaller amounts translocating to the fruit (1%). However, plants treated with MMA and DMA had higher arsenic concentrations in the shoots and fruit than those treated with arsenite or arsenate (Burlo et al. 1999). Terrestrial plants growing on land bordering arsenic-contaminated waters show relatively little arsenic content, even though the sediments have arsenic concentrations as high as 200 μg/g (Tamaki and Frankenberger 1992). Arsenic concentrations in vegetables grown in uncontaminated soils and contaminated soils containing arsenic, as well as other metals and organic contaminants, were generally <12 μg/kg wet weight. A maximum arsenic concentration of 18 μg/kg wet weight was found in unpeeled carrots grown in soil, which contained a mean arsenic concentration of 27 mg/kg dry weight (Samsøe-Petersen et al. 2002).

In a study by Rahman et al. (2004), CCA-treated lumber was used to construct raised garden beds to determine how far the components of CCA migrated in the soil and the uptake of these components by crops grown in the soil. Arsenic was found to diffuse laterally into the soil from the CCA-treated wood, with the highest concentrations found at 0–2 cm from the treated wood and a steady decline in

concentration with increased distance. The highest average arsenic concentrations found in soil closest (0–2 cm) to the CCA-treated wood were 56 and 46 μg/g in loamy sand and sandy loam soils, respectively. At a distance of 30–35 cm from the CCA-treated wood, arsenic concentrations were approximately 7 μg/g in both soils. All samples were of the top 0–15 cm of soil. Crops grown in both soil types within 0–2 cm of the CCA-treated wood contained higher concentrations of arsenic, 0.186 and 10.894 μg/g for carrots without peal and bean leaves and stems, respectively, than those grown at 1.5 m from the CCA-treated wood, 0.006 and 0.682 μg/g for bean pods and bean leaves and stems, respectively. However, based on FDA guidelines on tolerance limits, these crops would be considered approved for human consumption. Studies by Chirenje et al. (2003a) also showed that elevated arsenic concentrations were found in surface (0–5 cm) soils immediately surrounding, within the first 0.3 m, of utility poles, fences, and decks made with CCA-treated wood. Factors such as the preservative formula, fixation temperature, post treatment handling, and timber dimensions of CCA-treated wood, as well as the pH, salinity, and temperature of the leaching media can affect the leach rates from CCA-treated wood (Hingston et al. 2001). Studies of leaching of the components of CCA- and ACZA-treated wood used to construct a boardwalk in wetland environments reported elevated arsenic levels in soil and sediment below and adjacent to these structures. Generally, these levels decreased with increasing distance from the structure (Lebow et al. 2000). Increased concentrations of arsenic were also observed under CCA-treated bridges. Arsenic levels declined with distance from the bridge and were near background levels at 1.8–3 m from the bridge's perimeter (USDA/USDT 2000).

In a study by Lebow et al. (2003), the use of a water repellent finish on CCA-treated wood significantly reduces the amount of arsenic, as well as copper and chromium, in the run-off water. It was also observed the exposure to UV radiation caused a significant increase in leaching from both finished and unfinished samples of CCA-treated wood. Small amounts of arsenic can be transferred from CCA-treated wood to skin from touching CCA-treated wood surfaces (Hemond and Solo-Gabriele 2004; Kwon et al. 2004; Shalat et al. 2006; Ursitti et al. 2004; Wang et al. 2005).

Breslin and Adler-Ivanbrook (1998) examined the leaching of the copper, chromium, and arsenic from CCA-treated wood in laboratory studies using samples of treated southern yellow pine in solutions simulating estuarine waters. The tank leaching solutions were frequently sampled and replaced to approximate field conditions. Initial 12-hour fluxes ranging from 0.2×10^{-10} to 5.2×10^{-10} mol/mm^2 d was reported for arsenic. After 90 days, arsenic fluxes decreased to 0.5×10^{-11}–3.1×10^{-11} mol/mm^2 d. A study by Cooper (1991) demonstrated that the buffer system used in leaching studies of components from CCA-treated wood can significantly change the amount arsenic released from treated wood. Samples of four

species of CCA-treated wood were exposed to four acidic leaching solutions. In the samples exposed to water adjusted to pHs of 3.5, 4.5, and 5.5, losses of arsenic after 13 days were generally <7%. However, when a leaching solution of sodium hydroxide and citric acid buffer (pH 5.5) was used, the percent of arsenic leached ranged from 27.4 to 46.7% (Cooper 1991).

Arsenic bioaccumulation depends on various factors, such as environmental setting (marine, estuarine, freshwater), organism type (fish, invertebrate), trophic status within the aquatic food chain, exposure concentrations, and route of uptake (Williams et al. 2006). Bioaccumulation refers to the net accumulation of a chemical by aquatic organisms as a result of uptake from all environmental sources, such as water, food, and sediment, whereas bioconcentration refers to the uptake of a chemical by an aquatic organism through water (EPA 2003b). Biomagnification in aquatic food chains does not appear to be significant (EPA 1979, 1982b, 1983e, 2003b; Mason et al. 2000; Williams et al. 2006).

Bioconcentration of arsenic occurs in aquatic organisms, primarily in algae and lower invertebrates. Both bottom-feeding and predatory fish can accumulate contaminants found in water. Bottom-feeders are readily exposed to the greater quantities of metals, including the metalloid arsenic, which accumulate in sediments. Predators may bioaccumulate metals from the surrounding water or from feeding on other fish, including bottom-feeders, which can result in the biomagnification of the metals in their tissues. An extensive study of the factors affecting bioaccumulation of arsenic in two streams in western Maryland in 1997–1998 found no evidence of biomagnification since arsenic concentrations in organisms tend to decrease with increasing tropic level (Mason et al. 2000). Arsenic is mainly accumulated in the exoskeleton of invertebrates and in the livers of fish. No differences were found in the arsenic levels in different species of fish, which included herbivorous, insectivorous, and carnivorous species. The major bioaccumulation transfer is between water and algae, at the base of the food chain and this has a strong impact on the concentration in fish. National Contaminant Biomonitoring data produced by the Fish and Wildlife Service were used to test whether differences exist between bottom-feeders and predators in tissue levels of metals and other contaminants. No differences were found for arsenic (Kidwell et al. 1995). The bioconcentration factors (BCFs) of bryophytes, invertebrates, and fish (livers) in Swedish lakes and brooks impacted by smelter emissions were 8,700, 1,900–2,200, and 200–800, respectively (Lithner et al. 1995). EPA (2003b) assessed a large dataset of bioaccumulation data for various fish and invertebrate species. BCF values in this dataset ranged from 0.048 to 1,390.

Williams et al. (2006) reviewed 12 studies of arsenic bioaccumulation in freshwater fish, and proposed that BCF and bioaccumulation factor (BAF) values are not constant across arsenic concentrations in

water. BCF or BAF values from these 12 studies ranged from 0.1 to 3,091. Williams et al. (2006) found that BCF and BAF values appear to be the highest within the range of ambient arsenic concentrations, and decline steeply to relatively low levels as the arsenic concentrations in water increase. Based on this analysis, arsenic concentrations in tissue and BAF values may be a power function of arsenic concentrations in water. EPA (2007b) also reported that for many nonessential metals, including arsenic, accumulation is nonlinear with respect to exposure concentration.

6.3.2 Transformation and Degradation

6.3.2.1 Air

Arsenic is released into the atmosphere primarily as arsenic trioxide or, less frequently, in one of several volatile organic compounds, mainly arsines (EPA 1982b). Trivalent arsenic and methyl arsines in the atmosphere undergo oxidation to the pentavalent state (EPA 1984a), and arsenic in the atmosphere is usually a mixture of the trivalent and pentavalent forms (EPA 1984a; Scudlark and Church 1988). Photolysis is not considered an important fate process for arsenic compounds (EPA 1979).

6.3.2.2 Water

Arsenic in water can undergo a complex series of transformations, including oxidation-reduction reactions, ligand exchange, precipitation, and biotransformation (EPA 1979, 1984a; Sanders et al. 1994; Welch et al. 1988). Rate constants for these various reactions are not readily available, but the factors most strongly influencing fate processes in water include Eh, pH, metal sulfide and sulfide ion concentrations, iron concentrations, temperature, salinity, distribution and composition of the biota, season, and the nature and concentration of natural organic matter (EPA 1979; Farago 1997; Redman et al. 2002; Wakao et al. 1988). Organic arsenical pesticides, such as MSMA, DSMA, and DMA do not degrade by hydrolysis or by aquatic photolysis (EPA 2006). No formation of arsine gas from marine environments has been reported (Tamaki and Frankenberger 1992).

Inorganic species of arsenic are predominant in the aquatic environment. In the pH range of natural waters, the predominant aqueous inorganic As(V) species are the arsenate ions, $H_2AsO_4^-$ and $HAsO_4^{2-}$; the predominant inorganic As(III) species is $As(OH)_3$ (Aurillo et al. 1994; EPA 1982c). As(V) generally dominates in oxidizing environments such as surface water and As(III) dominates under reducing conditions such as may occur in groundwater containing high levels of arsenic. However, the reduction of arsenate to arsenite is slow, so arsenate can be found in reducing environments. Conversely, the oxidation of arsenite in oxidizing environments is moderately slow (half-life, 0.4–7 days in coastal

systems) and therefore, arsenite can be found in oxidizing environments (Mariner et al. 1996; Sanders et al. 1994). The main organic species in fresh water are MMA and DMA; however, these species are usually present at lower concentrations than inorganic arsenic species (Eisler 1994). (The toxicities of MMA and DMA are discussed in Chapter 3.) Aquatic microorganisms may reduce the arsenate to arsenite, as well as methylate arsenate to its mono- or dimethylated forms (Aurillo et al. 1994; Benson 1989; Braman and Foreback 1973; Edmonds and Francesconi 1987; Sanders et al. 1994). Methylated species are also produced by the biogenic reduction of more complex organoarsenic compounds like arsenocholine or arsenobetaine. Water samples from a number of lakes and estuaries, mostly in California, show measurable concentrations of methylated arsenic (equivalent to 1–59% of total arsenic) (Anderson and Bruland 1991). Within the oxic photic zone, arsenate and DMA were the dominant species. A seasonal study of one lake demonstrated that DMA was the dominant form of arsenic in surface waters during late summer and fall. Methylated species declined and arsenate species increased when the lake turned over in late fall. Mono Lake, a highly alkaline body of water, and four rivers did not have measurable concentrations of methylated arsenic. It was hypothesized that the reason why methylated forms were not detected in Mono Lake was that the extremely high inorganic arsenic concentrations in the lake, 230 μM (17 mg/L), could overwhelm the analysis of small amounts of organic forms. Other possibilities are that the high alkalinity or very high phosphate levels in the water, 260 μM (25 mg/L), are not conducive to biogenic methylation (Anderson and Bruland 1991). Both reduction and methylation of As(V) may lead to increased mobilization of arsenic, since As(III), dimethylarsinates, and monomethylarsonates are much less particle-reactive than As(V) (Aurillo et al. 1994). In the estuarial Patuxet River, Maryland, arsenate concentrations peaked during the summer, at 1.0 μg/L in 1988–1989 (Sanders et al. 1994). In contrast, winter to spring levels were around 0.1 μg/L. Arsenite concentrations were irregularly present at low levels during the year. Peaks of DMA occurred at various times, particularly in the winter and late spring and appeared to be linked with algal blooms. The DMA peak declined over several months that was followed by a rise in MMA. The MMA was thought to be occurring as a degradation product of DMA. A similar seasonal pattern of arsenic speciation was observed in Chesapeake Bay. Arsenite methylation took place during the warmer months leading to changes down the main stem of the bay; arsensite production dominated the upper reaches of the bay and methylated species dominated the more saline lower reaches. In coastal waters, reduced and methylated species are present in lower concentrations, around 10–20% of total arsenic (Sanders et al. 1994). In groundwater, arsenic generally exists as the oxyanion of arsenate ($H_xAsO_4^{3-x}$) or arsenite ($H_xAsO_3^{3-x}$), or both; however, the distribution between arsenite and arsenate is not always predictable based on oxidation-reduction potential (Robertson 1989; Welch et al. 1988).

6.3.2.3 Sediment and Soil

In soil, arsenic is found as a complex mixture of mineral phases, such as co-precipitated and sorbed species, as well as dissolved species (Roberts et al. 2007). The degree of arsenic solubility in soil will depend on the amount of arsenic distributed between these different mineral phases. The dissolution of arsenic is also affected by particle size. The distribution between these phases may reflect the arsenic source (e.g., pesticide application, wood treatment, tanning, or mining operations), and may change with weathering and associations with iron and manganese oxides and phosphate minerals in the soil (Roberts et al. 2007; Ruby et al. 1999). Davis et al. (1996) reported that in soil in Anaconda, Montana, a smelting site from 1860 to 1980, contained arsenic that is only in a sparingly soluble form, consisting of primarily arsenic oxides and phosphates.

The arsenic cycle in soils is complex, with many biotic and abiotic processes controlling its overall fate and environmental impact. Arsenic in soil exists in various oxidation states and chemical species, depending upon soil pH and oxidation-reduction potential. Under most environmental conditions, inorganic As(V) will exist as a mixture of arsenate anions, $H_2AsO_4^-$ and $HAsO_4^{2-}$, and inorganic As(III) will exist as H_3AsO_3. The arsenate and arsenite oxyanions have various degrees of protonation depending upon pH (EPA 1982b; McGeehan 1996). As(V) predominates in aerobic soils, and As(III) predominates in slightly reduced soils (e.g., temporarily flooded) or sediments (EPA 1982b; Sanders et al. 1994). As(III) commonly partitions to the aqueous phase in anoxic environments, and would be more mobile. As(V) usually remains bound to minerals, such as ferrihydrite and alumina, limiting its mobility and bioavailability (Rhine et al. 2006).

Arsenite is moderately unstable in the presence of oxygen; however, it can be found under aerobic conditions as well (Sanders et al. 1994). While arsenate is strongly sorbed by soils under aerobic conditions, it is rapidly desorbed as the system becomes anaerobic. Once it is desorbed, arsenate can be reduced to arsenite, which exhibits greater mobility in soils (McGeehan 1996). Transformations between the various oxidation states and species of arsenic occur as a result of biotic or abiotic processes (Bhumbla and Keefer 1994). While degradation of an organic compound is typically considered complete mineralization, in the case of organic arsenic compounds, the element arsenic itself cannot be degraded. However, the organic portion of the molecule can be metabolized (Woolson 1976).

Arsenicals applied to soils may be methylated by microorganisms to arsines, which are lost through volatilization, and organic forms may be mineralized to inorganic forms. Gao and Burau (1997) reported

that the overall percentage of DMA and MMA minerialized after 70 days ranged from 3 to 87% in air-dry soil and a soil near saturation, respectively. The rate of demethylation of DMA increased with soil moisture. Over the same 70-day period, arsenic losses as volatile arsines were much lower than minerialization, ranging from 0.001 to 0.4%. Arsine evolution rates followed the order: DMA>MMA>arsenite=arsenate (Gao and Burau 1997). Woolson and Kearney (1973) reported that ^{14}C-labeled DMA degraded differently in soils under aerobic and anaerobic conditions. Under anaerobic conditions, 61% of the applied DMA was converted to a volatile alkyl arsine after 24 weeks, and lost from the soil system. Under aerobic conditions, 35% was converted to a volatile organo-arsenic compound, possibly dimethyl arsine, and 41% was converted to $^{14}CO_2$ and arsenate after 24 weeks. Similar to microorganisms in soils, Reimer (1989) reported that microorganisms found in natural marine sediments and sediments contaminated with mine-tailings are also capable of methylating arsenic under aerobic and anaerobic conditions. Von Endt et al. (1968) reported that the degradation of ^{14}C-labelled monosodium methanearsonate (MSMA) was found to range from 1.7 to 10% in Dundee silty clay loam soil and Sharkey clay soil after 60 days, respectively. MSMA decomposition to CO_2 was a slow process without a lag period. Sterilized soils were found to produce essentially no $^{14}CO_2$ (0.7%) after 60 days, indicating that soil bacteria contributed to the decomposition of MSMA (Von Endt et al. 1968). Akkari et al. (1986) studied the degradation of MSMA in various soils. At 20% water content, half-lives of 144, 88, and 178 days were reported in Sharkey clay, Taloka silt loam, and Steele-Crevasse sand loam, respectively. The Sharkey soil with the highest clay content was expected to have the greatest adsorptive capacity for both water and MSMA, reducing the amount of MSMA available in the soil solution to microorganisms that degrade the MSMA. The half-lives were 25, 41, and 178 days under anaerobic (flooded) conditions in Sharkey clay, Taloka silt loam, and Steele-Crevasse sand loam, respectively. Under flooded conditions, MSMA degradation occurs by reductive methylation to form arsinite and alkylarsine gas. The authors attributed the longer half-lives for MSMA degradation in the Steele-Crevasse sand loam soil to its low organic matter content, which may have supported fewer microbial populations needed for oxidation demethylation under aerobic conditions. Under flooded conditions, anaerobiosis is expected to be slowest in low organic matter sandy loam soils (Akkari et al. 1986).

Organic arsenical pesticides, such as MSMA, DSMA, and DMA, do not degrade by hydrolysis or by soil photolysis (EPA 2006).

Roxarsone (3-nitro-4-hydroxyphenylarsonic acid) used in poultry feed is found excreted unchanged in poultry litter (bedding and manure). Roxarsone found in poultry litter, which is used to amend agricultural soil, was found to degrade to arsenate in approximately 3–4 weeks upon composting

(Garbarino et al. 2003). In addition, the arsenic in poultry litter was found to be easily mobilized by water; however, its leach rate from amended soils was slow enough that it accumulated in soils (Rutherford et al. 2003).

A sequential fractionation scheme was used to assess the chemical nature, and thus the potential bioavailability, of arsenic at cattle dip sites in Australia where sodium arsenite was used extensively in cattle dips from the turn of the century until the early 1950s (McLaren et al. 1998). Most sites contained substantial amounts, 13% on the average, of arsenic in the two most labile fractions indicating a high potential for bioaccessibility and leaching. The bulk of the arsenic appeared to be associated with amorphous iron and aluminum minerals in soil. Similarly, arsenic in soil and mine waste in the Tamar Valley in England was found to be concentrated in a fraction associated with iron and organic-iron (Kavanagh et al. 1997). Laboratory studies were performed to assess the phase partitioning of trace metals, including the metalloid arsenic, to sediment from the Coeur d'Alene River, a mining area of Idaho, and the release of metals under simulated minor and major flooding events (LaForce et al. 1998). Arsenic was primarily associated with the iron and manganese oxides as seen by its large release when these oxides were reduced. Arsenic levels were comparatively low in the organic fraction and remaining residual fraction and negligible in the extractible fractions.

6.3.2.4 Other Media

Carbonell-Barrachina et al. (2000) found the speciation and solubility of arsenic in sewage sludge suspensions to be affected by pH and Eh. Under oxidizing conditions, the solubility of arsenic was low, with a major portion of the soluble arsenic present as organic arsenic compounds, mainly dimethylarsinic acid (approximately 74% of the total arsenic in solution). Under moderately reducing conditions (0–100 mV), inorganic arsenic accounted for the majority (90%) of the total arsenic in solution, and the solubility of arsenic was increased due to dissolution of iron oxyhydroxides. Under strongly reducing conditions (-250 mV), arsenic solubility was decreased by the formation of insoluble sulfides. The pH of the solution was also found to influence the speciation and solubility of arsenic. At neutral pH, the solubility of arsenic was at its maximum, and decreased under acidic or alkaline conditions. Inorganic arsenic species were the dominant species at pH 5.0; at pH 6.5, the major soluble forms were organic arsenic species. The biomethylation of arsenic was limited at acidic pH, and was at its maximum at near neutral pH (Carbonell-Barrachina et al. 2000).

6.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

Reliable evaluation of the potential for human exposure to arsenic depends in part on the reliability of supporting analytical data from environmental samples and biological specimens. Concentrations of arsenic in unpolluted atmospheres and in pristine surface waters are often so low as to be near the limits of current analytical methods. In reviewing data on arsenic levels monitored or estimated in the environment, it should also be noted that the amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable. The analytical methods available for monitoring arsenic in a variety of environmental media are detailed in Chapter 7.

6.4.1 Air

Arsenic in ambient air is usually a mixture of particulate arsenite and arsenate; organic species are of negligible importance except in areas of substantial methylated arsenic pesticide application or biotic activity (EPA 1984a). Mean levels in ambient air in the United States have been reported to range from <1 to 3 ng/m^3 in remote areas and from 20 to 30 ng/m^3 in urban areas (Davidson et al. 1985; EPA 1982c; IARC 1980; NAS 1977a). EPA conducted a modeling study with the Assessment System for Population Exposure Nationwide (ASPEN) in which estimates of emissions of hazardous air pollutants were used to estimate ambient concentrations (Rosenbaum et al. 1999). Using 1990 data to estimate total emissions of arsenic in the conterminous 48 states, excluding road dust or windblown dust from construction or agricultural tilling, the 25th percentile, median, and 75th percentile arsenic concentration were estimated to be 9, 20, and 30 ng/m^3, respectively. Maps illustrating the amount of toxic air pollutant emissions, including arsenic compounds, by county in 1996 for the 48 coterminous states of the United States as well as Puerto Rico and the Virgin Islands are available on the internet at http://www.epa.gov/ttn/atw/-nata/mapemis.html, as of March 2005. Schroeder et al. (1987) listed ranges of arsenic concentrations in air of 0.007–1.9, 1.0–28, and 2–2,320 ng/m^3 in remote, rural, and urban areas, respectively. The average annual arsenic concentration in air at Nahant, Massachusetts, just north of Boston, between September 1992 and September 1993, was 1.2 ng/m^3; 75% of the arsenic was associated with fine (<2.5 μm) particles. The long-term means of the ambient concentrations of arsenic measured in urban areas of the Great Lakes region from 1982 to 1993 ranged from 4.2 to 9.6 ng/m^3 (Pirrone and Keeler 1996). Large cities generally have higher arsenic air concentrations than smaller ones due to emissions from coal-fired power plants (IARC 1980), but maximum 24-hour concentrations generally are <100 ng/m^3 (EPA 1984a). In the spring of 1990, aerosols and cloud water that were sampled by aircraft at an altitude of 1.2–3 km above the Midwestern United States had a mean mixed layer arsenic concentration of 1.6±0.9 ng/m^3 (Burkhard et al. 1994). A mean arsenic concentration of 1.0±0.5 ng/m^3 was reported at

Mayville, New York, a site 400 km to the northwest of the sampling area and directly downwind on most days.

Arsenic was monitored at an application site in the San Joaquin Valley, California and at four sites in nearby communities in 1987 where sodium arsenite was used as a fungicide on tokay grapes (Baker et al. 1996). The maximum arsenic concentration measured 15–20 meters from the edge of the field was 260 ng/m^3. The maximum arsenic concentration at four community sites in the area was 76 ng/m^3. The concentration at an urban background site was 3 ng/m^3 (Baker et al. 1996). Sodium arsenite is no longer registered in California (Baker et al. 1996). The highest historic arsenic levels detected in the atmosphere were near nonferrous metal smelters, with reported concentrations up to 2,500 ng/m^3 (IARC 1980; NAS 1977a; Schroeder et al. 1987).

Arsenic air concentrations measured in several indoor public places (e.g., cafeteria, coffee house, music club, Amtrak train, and several restaurants) with environmental tobacco smoke (ETS) ranged from <0.1 to 1 ng/m^3, with a mean of 0.4±0.3 ng/m^3. Sites that were ETS-free (university office and library) had arsenic concentrations <0.13 ng/m^3 (Landsberger and Wu 1995). The Toxic Exposure Assessment at Columbia/Harvard (TEACH) study measured levels of various toxics in New York City air in 1999. Exposures were assessed in a group of 46 high school students in West Central Harlem. Mean arsenic concentrations in summer home outdoor, home indoor, and personal air of the participants were 0.37, 0.40, and 0.45 ng/m^3, respectively (Kinney et al. 2002). Detected arsenic concentrations in indoor and outdoor air collected as part of the National Human Exposure Assessment Survey (NHEXAS) in Arizona ranged from 3.4 to 22.3 and from 3.5 to 25.7 ng/m^3, respectively, with 71 and 68% below the detection limit (1.8–14.3 ng/m^3) (O'Rourke et al. 1999).

6.4.2 Water

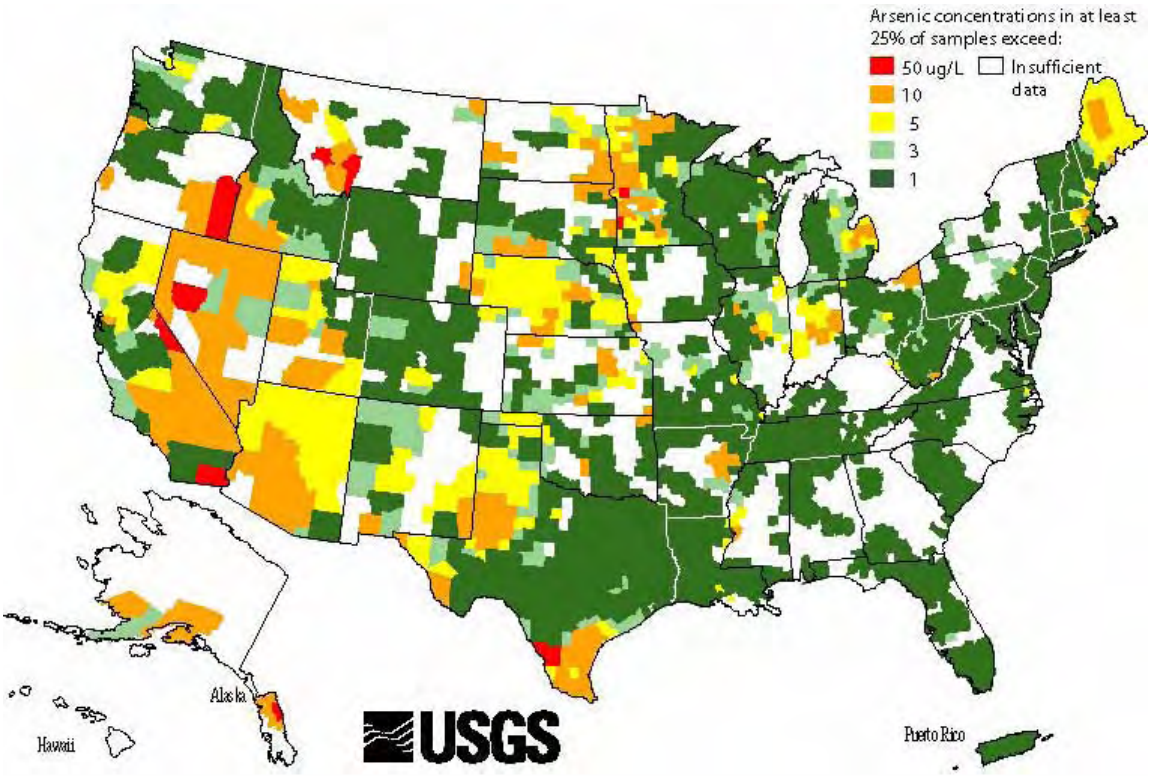
Arsenic is widely distributed in surface water, groundwater, and finished drinking water in the United States. A survey of 293 stations in two nationwide sampling networks on major U.S. rivers found median arsenic levels to be 1 μg/L; the 75th percentile level was 3 μg/L (Smith et al. 1987). Arsenic was detected in 1,298 of 3,452 surface water samples recorded in the STORET database for 2004 at concentrations ranging from 0.138 to 1,700 μg/L in samples where arsenic was detected (EPA 2005c). Two streams in western Maryland that were the focus of a major bioaccumulation study in 1997–1998 had arsenic concentrations of 0.370±0.200 and 0.670±0.460 μg/L (Mason et al. 2000). Surface water will be impacted by runoff from polluted sites. An average arsenic concentration of 5.12 μg/L was reported in

water from Moon Lake, a Mississippi River alluvial floodplain in northwest Mississippi. Intensive cultivation has occurred in this area, including cotton, soybeans, and rice (Cooper and Gillespie 2001). Hard-rock mining activities occurred in the southern part of Colorado and New Mexico north of Taos since the latter part of the 19th century until recently, which have impacted the Rio Grande and its tributaries. A mean arsenic concentration of approximately 0.8 μg/L was reported for the main stem of the Rio Grande sampled in June and September 1994. Arsenic concentrations in the Alamosa River, Colorado were 0.11 and 0.14 μg/L in June and September 1994, respectively, and 1.4 μg/L in Big Arsenic Spring, New Mexico in September 1994 (Taylor et al. 2001). Arsenic concentrations in water from watersheds in Black Hills, South Dakota, an area impacted by gold mining activities ranged from 2.5 to 55 μg/L and from 1.7 to 51 μg/L in unfiltered and filtered samples, respectively; concentrations from reference areas ranged from 1.1 to 3.4 μg/L and from 0.9 to1.9 μg/L in unfiltered and filtered samples, respectively (May et al. 2001). Arsenic concentrations ranged from 0.29 to 34.0 μg/L in water samples from Wakulla River and St. Joseph Bay North, along the Florida Panhandle; arsenic contamination in this area is likely to result from nonpoint source pollution (Philp et al. 2003).

Data on total arsenic in surface water from a number of seas and oceans show levels of <1 μg/L, except in the Antarctic Ocean and Southwest Pacific Oceans where the levels are 1.1 and 1.2 μg/L, respectively. Levels in coastal waters and estuaries are generally somewhat higher, in the range of 1–3 μg/L. However, estuarine water in Salinas, California had arsenic levels of 7.42 μg/L (Francesconi et al. 1994). The dissolved arsenic concentration in water at 40 sites in the Indian River Lagoon System in Florida ranged from 0.35 to 1.6 μg/L with a mean of 0.89±0.34 μg/L (Trocine and Trefry 1996). Thermal waters generally have arsenic levels of 20–3,800 μg/L, although levels as high as 276,000 μg/L have been recorded (Eisler 1994).

Arsenic levels in groundwater average about 1–2 μg/L, except in some western states with volcanic rock and sulfidic mineral deposits high in arsenic, where arsenic levels up to 3,400 μg/L have been observed (IARC 1980; Page 1981; Robertson 1989; Welch et al. 1988). In western mining areas, groundwater arsenic concentrations up to 48,000 μg/L have been reported (Welch et al. 1988). Arsenic concentrations in groundwater samples collected form 73 wells in 10 counties in southeast Michigan in 1997 ranged from 0.5 to 278 μg/L, with an average of 29 μg/L. Most (53–98%) of the arsenic was detected as arsenite (Kim et al. 2002). The U.S. Geological Survey mapped concentrations of arsenic in approximately 31,350 groundwater samples collected between 1973 and 2001; the counties in which at least 25% of wells exceed various levels are shown in Figure 6-2 (USGS 2007a). Most arsenic in natural waters is a

Figure 6-2. Counties in Which at Least 25% of Wells Exceed Different Arsenic Levels



Source: USGS 2007a

mixture of arsenate and arsenite, with arsenate usually predominating (Braman and Foreback 1973; EPA 1982c, 1984a). Methylated forms have also been detected in both surface water and groundwater, at levels ranging from 0.01 to 7.4 μg/L (Braman and Foreback 1973; Hood 1985), with most values below 0.3 μg/L (Hood 1985). In a survey of shallow groundwater quality in the alluvial aquifer beneath a major urban center, Denver, Colorado, arsenic levels in the 30 randomly-chosen wells sampled had median levels of <1 μg/L; the maximum level was 33 μg/L (Bruce and McMahon 1996). Arsenic levels in groundwater sometimes exceeded the EPA maximum contaminant level (MCL), which was 50 μg/L at the time, in the Willamette Valley, Oregon and a nine-county region of southeastern Michigan (USGS 1999b, 1999c).

Arsenic has also been detected in rainwater at average concentrations of 0.2–0.5 μg/L (Welch et al. 1988). This range is consistent with that found in a 1997–1998 study in western Maryland, which was the focus of a major bioaccumulation study (Mason et al. 2000). Arsenic levels in wet deposition in the watershed as well as throughfall into the two streams were 0.345±0.392, 0.400±0.400, and 0.330±0.250 μg/L, respectively. Median arsenic concentrations in 30-day rainwater composite samples collected May-September 1994 from eight arctic catchments in northern Europe at varying distances and wind directions from the emissions of a Russian nickel ore mining, roasting, and smelting industry on the Kola Peninsula ranged from 0.07 to 12.3 μg/L (Reimann et al. 1997). Rain and snow samples were collected during the fall of 1996 and winter of 1997 at eight locations in a semi-circular pattern radiating out (2–15 km) in the direction of the prevailing wind from the Claremont incinerator located in New Hampshire. This incinerator processes 200 tons of solid waste per day. Arsenic concentrations in rainwater and snow ranged from 0.020 to 0.079 μg/L and from 0.80 to 1.28 μg/L, respectively (Feng et al. 2000).

Drinking water is one of the most important sources of arsenic exposure. Surveys of drinking water in the United States have found that >99% of public water supplies have arsenic concentrations below the EPA MCL, which was 50 μg/L at the time (EPA 1984a). In an EPA study of tap water from 3,834 U.S. residences, the average value was 2.4 μg/L (EPA 1982c).

Before the MCL for arsenic in drinking water was lowered from 50 to 10 μg/L, studies were undertaken to ascertain how different standards would affect compliance. One such survey sponsored by the Water Industry Technical Action Fund was the National Arsenic Occurrence Survey (NAOS). NAOS was based on a representational survey of public water systems defined by source type, system size, and geographical location. Additionally, it included a natural occurrence factor, a stratifying variable that could qualitatively describe the likelihood of arsenic occurrence in the supply. To predict finished water

arsenic concentrations, data on the water treatment options, efficiency, and frequency of use were factored in. The results of the NAOS are presented in Table 6-3. The NAOS results are in general agreement with two older and more limited national surveys, EPA's National Inorganics and Radionuclides Survey (NIRS) and the Metropolitan Water District of Southern California Survey (MWDSC). The percentages of water systems that would be out of compliance are estimated to be 1.7, 3.6, 9.3, and 20.7% for arsenic MCLs of 20, 10, 5, and 2 μg/L, respectively. Arsenic concentrations were determined in drinking in EPA Region V (Indiana, Illinois, Michigan, Minnesota, Ohio, and Wisconsin) as part of the NHEXAS; mean arsenic concentration in flushed and standing tap water were both 1.1 μg/L (Thomas et al. 1999). A review by Frost et al. (2003) of existing data from the EPA Arsenic Occurrence and Exposure Database, as well as additional data from state health and environmental departments and water utilities found that 33 counties in 11 states had estimated mean drinking water arsenic concentrations of 10 μg/L or greater. Eleven counties had mean arsenic concentrations of ≥20 μg/L, and two counties had mean arsenic concentrations of ≥50 μg/L (Frost et al. 2003).

The north central region and the western region of the United States have the highest arsenic levels in surface water and groundwater sources, respectively. In a study of drinking water from New Hampshire, arsenic concentrations ranged from <0.01 to 180 μg/L in the 793 households tested. More than 10% of the private wells had arsenic concentrations >10 μg/L, and 2.5% had levels >50 μg/L (Karagas et al. 1998). In New Hampshire, 992 randomly selected household water samples were analyzed for arsenic levels and the results for domestic well users were compared with those for users of municipal water supplies (Peters et al. 1999). The concentrations ranged from <0.0003 to 180 μg/L, with water from domestic wells containing significantly more arsenic than water from municipal supplies; the median concentration of the former was about 0.5 μg/L and the latter was 0.2 μg/L. None of the municipal supplies exceeded an arsenic concentration of 50 μg/L, and 2% of the domestic wells were found to have arsenic concentrations that exceeded 50 μg/L. Approximately 2% of the municipal water users have water with arsenic levels exceeding 10 μg/L compared with 13% of domestic wells. Twenty-five percent of domestic wells and 5% of municipal supplies were found to have arsenic concentrations exceeding 2 μg/L. The highest arsenic levels in New Hampshire are associated with bedrock wells in the south eastern and south central part of the state (Peters et al. 1999). In a study of arsenic in well water supplies in Saskatchewan, Canada, 13% of samples were >20 μg/L and one sample exceeded 100 μg/L (Thompson et al. 1999). It was noted that the samples with high arsenic levels were derived from sites that were in near proximity to each other, indicating the presence of 'hot spots' with similar geological characteristics. As part of an epidemiological study, Engel and Smith (1994) investigated the levels of arsenic in drinking

Table 6-3. Regional Occurrence of Arsenic in U.S. Water Sources and Finished Drinking Water

	Arsenic concentration in μg/L			
Geographical region	<1	1–5	5–20	>20
Occurrence in U.S. surface water sources				
Region 1. New England	50	50	0	0
Region 2. Mid-Atlantic	84	12	4	0
Region 3. South East	93	7	0	0
Region 4. Midwest	24	76	0	0
Region 5. South Central	32	55	13	0
Region 6. North Central	33	22	33	0
Region 7. Western	42	58	0	0
Occurrence in U.S. groundwater sources				
Region 1. New England	71	21	7	0
Region 2. Mid-Atlantic	81	4	11	4
Region 3. South East	82	14	2	0
Region 4. Midwest	40	40	15	5
Region 5. South Central	68	27	15	0
Region 6. North Central	30	40	30	0
Region 7. Western	24	34	28	14
Occurrence in U.S. finished surface water supplies				
Region 1. New England	88	12	0	0
Region 2. Mid-Atlantic	92	8	0	0
Region 3. South East	100	0	0	0
Region 4. Midwest	73	27	0	0
Region 5. South Central	74	19	7	0
Region 6. North Central	44	44	0	12
Region 7. Western	42	58	0	0
Occurrence in U.S. finished groundwater supplies				
Region 1. New England	79	21	0	0
Region 2. Mid-Atlantic	81	4	11	4
Region 3. South East	94	4	2	0
Region 4. Midwest	58	27	12	3
Region 5. South Central	61	27	12	0
Region 6. North Central	40	50	10	0
Region 7. Western	20	40	22	12

Source: National Arsenic Occurrence Survey (Frey and Edwards 1997)

water throughout the United States between 1968 and 1984. They found that 30 counties in 11 states had mean arsenic levels of >5 μg/L, with a range of 5.4–91.5 μg/L; 15 counties had mean levels from 5 to 10 μg/L; 10 counties had mean levels from 10 to 20 μg/L; and 5 counties had levels >20 μg/L. The highest levels were found in Churchill County, Nevada, where 89% of the population was exposed to a mean arsenic concentration of 100 μg/L and 11% to a mean of 27 μg/L. A study by Frost et al. (2003) identified 33 counties from 11 states in which the average arsenic concentration of at least 75% of public wells was >10 μg/L. Arsenic concentrations in drinking water from these counties ranged from 10.3 to 90.0 μg/L in Pinal, Arizona and Churchill, Nevada, respectively (Frost et al. 2003).

Many communities have high levels of arsenic in their drinking water because of contamination or as a result of the geology of the area. In Millard County, Utah, seven towns had median and maximum arsenic levels of 18.1–190.7 and 125–620 μg/L, respectively, in their drinking water (Lewis et al. 1999). The mean arsenic concentration in tap water from homes in Ajo, Arizona, about 2 miles from an open pit copper mine and smelter was 90 μg/L (Morse et al. 1979). The town's water was supplied from five deep wells.

Countries such as Mexico, Bangladesh, India, Chile, Argentina, and Vietnam have highly elevated levels of arsenic in drinking water in some regions (Bagla and Kaiser 1996; Berg et al. 2001; Tondel et al. 1999; WHO 2001; Wyatt et al. 1998a, 1998b). In Bangladesh and West Bengal, the soil naturally contains high levels of arsenic, which leaches into the shallow groundwater that is tapped for drinking water. In West Bengal, India, it is estimated that more than one million Indians are drinking arsenic-laced water and tens of millions more could be at risk in areas that have not been tested for contamination. Analysis of 20,000 tube-well waters revealed that 62% have arsenic at levels above the World Health Organization (WHO) permissible exposure limit (PEL) in drinking water of 10 μg/L, with some as high as 3,700 μg/L (Bagla and Kaiser 1996). Analysis of 10,991 and 58,166 groundwater samples from 42 and 9 arsenic-affected districts in Bangladesh and West Bengal were found to have arsenic levels that were 59 and 34%, respectively, above 50 μg/L (Chowdhury et al. 2000). Berg et al. (2001), studied the arsenic contamination of the Red River alluvial tract in Hanoi, Vietnam and the surrounding rural areas. Arsenic concentrations in groundwater from private small-scale tube-wells averaged 159 μg/L, ranging from 1 to 3,050 μg/L. Arsenic concentrations ranged from 37 to 320 μg/L in raw groundwater pumped from the lower aquifer for the Hanoi water supply (Berg et al. 2001). Several investigators have noticed a correlation between high levels of arsenic and fluoride in drinking water (Wyatt et al. 1998a, 1998b). Arsenic concentrations in drinking water from four villages in Bangladesh ranged from 10 to 2,040 μg/L (Tondel et al. 1999).

6.4.3 Sediment and Soil

Arsenic is widely distributed in the Earth's crust, which contains about 3.4 ppm arsenic (Wedepohl 1991). It is mostly found in nature minerals, such as realgar (As_4S_4), orpiment (As_2S_3), and arsenolite (As_2O_3), and only found in its elemental form to a small extent. There are over 150 arsenic-bearing minerals (Budavari et al. 2001; Carapella 1992). Arsenic concentrations in soils from various countries can range from 0.1 to 50 μg/g and can vary widely among geographic regions. Typical arsenic concentrations for uncontaminated soils range from 1 to 40 μg/g, with the lowest concentrations in sandy soils and soils derived from granites. Higher arsenic concentrations are found in alluvial soils and soils with high organic content (Mandal and Suzuki 2002). Arsenic in soil may originate from the parent materials that form the soil, industrial wastes, or use of arsenical pesticides. Geological processes that may lead to high arsenic concentrations in rock and subsequently the surrounding soil include hydrothermic activity and pegmatite formation (Peters et al. 1999). In the first case, thermal activity results in the dissolution and transport of metals, including the metalloid arsenic, which are precipitated in fractures in rocks. In the second process, cooling magmas may concentrate metals that are injected into rocks, crystallizing as pegmatites. Areas of volcanic activity include large areas of California, Hawaii, Alaska, Iceland, and New Zealand.

The U.S. Geological Survey reports the mean and range of arsenic in soil and other surficial materials as 7.2 and <0.1–97 μg/g, respectively (USGS 1984). The concentrations of arsenic in 445 Florida surface soils ranged from 0.01 to 50.6 μg/g (Chen et al. 1999). The median, arithmetic mean, and geometric mean were 0.35, 1.34±3.77, and 0.42±4.10 μg/g, respectively. Chirenje et al. (2003b) reported a geometric mean arsenic concentrations of 0.40 (0.21–660) and 2.81 (0.32–110) μg/g in surface soil samples (0–20 cm) collected in May–June 2000 from Gainesville and Miami, Florida, respectively. The geometric mean arsenic concentration in 50 California soils was 2.8 μg/g (Chen et al. 1999). In the Florida surface soils, arsenic was highly correlated (α=0.0001) with the soil content of clay, organic carbon, CEC, total iron, and total aluminum. Arsenic tends to be associated with clay fractions and iron and manganese oxyhydroxides. Soils of granitic origin are generally low in arsenic, about 4 μg/g, whereas arsenic in soils derived from sedimentary rocks may be as high as 20–30 μg/g (Yan-Chu 1994). Soils overlying arsenic-rich geologic deposits, such as sulfide ores, may have soil concentrations two orders of magnitude higher (NAS 1977a).

Soils in mining areas or near smelters may contain high levels of arsenic. Arsenic concentrations up to 27,000 μg/g were reported in soils contaminated with mine or smelter wastes (EPA 1982b). Soils at an abandoned mining site in the Tamar Valley in southwest England have arsenic concentrations that may exceed 50,000 μg/g (Erry et al. 1999). The average arsenic levels in the top 2 cm of different soil types in the vicinity of a former copper smelter in Anaconda, Montana, ranged from 121 to 236 μg/g; levels were significantly related to proximity and wind direction to the smelter site (Hwang et al. 1997a). Smelter fallout can contaminate land miles from the source. Soils in mainland southern King County were studied for the presence of arsenic and lead (WSDOE 2005). Soil samples were collected in the fall of 1999 and the spring of 2001 from locations around the ASARCO smelter, which operated in Ruston from the 1890s to 1986. The study area ran roughly from the I-90 corridor south to the King-Pierce county line, from the Puget Sound shore to the Cascade foothills. Almost all of the contamination was found was in the 0–6-inch depths of the cores samples; 62 of the 75 samples were found to have arsenic levels above 20 ppm (WSDOE 2005).

Soil on agricultural lands treated with arsenical pesticides may retain substantial amounts of arsenic. One study reported an arsenic concentration of 22 μg/g in treated soil compared to 2 μg/g for nearby untreated soil (EPA 1982b). Arsenic was measured in soil samples taken from 10 potato fields in Suffolk County on Long Island, New York, where sodium arsenite had been used for vine control and fall weed control for many years. Lead arsenate also may have been used as an insecticide in certain areas. The mean arsenic levels taken at a depth of 0–18 cm from each of the 10 fields ranged from 27.8±5.44 μg/g dry weight (n=10) to 51.0±7.40 μg/g dry weight (n=10). These levels were markedly higher than the level of 2.26±0.33 μg/g (n=10) for untreated control soils (Sanok et al. 1995). A survey was conducted in 1993 to determine the concentrations of arsenic and lead in soil samples from 13 old orchards in New York State. Lead arsenate was used for pest control in fruit orchards for many years, mainly from the 1930s to 1960s, and residues remain in the soil. Concentrations of arsenic ranged from 1.60 to 141 μg/g dry weight (Merwin et al. 1994). Arsenic and lead concentrations were also measured in former orchard soils contaminated by lead arsenate from the Hanford site in Washington State. The mean arsenic concentration in surface (5–10 cm) and subsurface (10–50 cm) soils were 30 (2.9–270) and 74 (32–180) μg/g dry weight, respectively (Yokel and Delistraty 2003). Average arsenic concentration of 5.728, 5.614, and 6.746 μg/g were reported in soils, lake sediments, and wetland sediments, respectively, from Moon Lake, a Mississippi River alluvial floodplain in northwest Mississippi. Intensive cultivation has occurred in this area, including cotton, soybeans, and rice (Cooper and Gillespie 2001). A geometric mean arsenic concentration of 20.6 mg/kg (range 4.6–340 mg/kg) was reported soil collected during the

summer and fall of 2003 from 85 homes in Middleport, New York, where historical pesticide manufacturing was associated with arsenic in the soil (Tsuji et al. 2005).

The Washington State Area-wide Soil Contamination Project provides various data on arsenic contamination in soils across Washington State (Washington State 2006). Arsenic concentrations within areas affected by area-wide soil contamination are highly variable, ranging from natural background levels to >3,000 ppm in smelter areas. Generally, average arsenic concentrations in soil at developed properties are <100 ppm. Areas affected by smelter emissions in King, Pierce, Snohomish, and Stevens Counties have a higher likelihood of arsenic soil contamination than other areas of the State due to historical emissions from metal smelters located in Tacoma, Harbor Island, Everett, Northport, and Trail, British Columbia. Areas where apples and pears were historically grown, such as Chelan, Spokane, Yakima, and Okanogan Counties, also have a higher likelihood of arsenic soil contamination than other areas due to the past use of lead arsenate pesticides. Generally, arsenic contamination in soils from historical smelter emissions and historical use of lead-arsenate pesticides is found in the upper 6–18 inches of soil (Washington State 2006).

The New Jersey Department of Environmental Protection (Historic Pesticide Contamination Task Force 1999) reported on the analysis of soil samples collected from 18 sites for various pesticide residues, including arsenic, from current and former agricultural sites in New Jersey in order to assess contamination from historic pesticide use. Arsenic was detected in all 463 samples, with concentrations ranging from 1.4 to 310 ppm.

Natural concentrations of arsenic in sediments are usually <10 μg/g dry weight, but can vary widely around the world (Mandal and Suzuki 2002). Sediment arsenic concentrations reported for U.S. rivers, lakes, and streams range from about 0.1 to 4,000 μg/g (Eisler 1994; Heit et al. 1984; NAS 1977a; Welch et al. 1988). During August through November 1992 and August 1993, bed sediment in the South Platte River Basin (Colorado, Nebraska, and Wyoming) was sampled and analyzed for 45 elements, including arsenic. The range of arsenic found was 2.8–31 μg/g dry weight and the geometric mean (n=23) was 5.7 μg/g (Heiny and Tate 1997). The arsenic concentration in surface sediment (0–2 cm) at 43 sites in the Indian River Lagoon System in Florida ranged from 0.6 to 15 μg/g dry weight with a mean of 5.0±3.9 μg/g (Trocine and Trefry 1996). Arsenic levels were well correlated with those of aluminum. Correlation with aluminum levels is used to normalize sediment level concentrations to natural levels in Florida estuaries. Surficial sediments collected from 18 locations in 3 major tributaries to Newark Bay, New Jersey, were analyzed for 7 toxic metals, including arsenic (Bonnevie et al. 1994). The highest

concentrations of arsenic were found in the Rahway River adjacent to a chemical plant, 58 μg/g dry weight, and in the Hackensack River adjacent to a coal-fired power plant, 49 μg/g. The average arsenic concentration for all sediments was 17±16 μg/g. Sediments collected from seven sites in Baltimore Harbor, Maryland, at five seasonal periods between June 1987 and June 1988 had a geometric mean maximum of 7.29 μg/g dry weight and a geometric mean minimum of 1.25 μg/g (Miles and Tome 1997). This harbor is one of two sub-tributaries of the Chesapeake Bay where contaminants have been discharged on a large scale.

The upper Clark Fork River basin in western Montana is widely contaminated by metals from past mining, milling, and smelting activities. In a 1991 study, arsenic levels were determined in sediment along the river and in a reservoir 205 km downstream. Total arsenic in sediments from Clark Fork River decreased from 404 μg/g dry weight at the farthest upstream sampling station to 11 μg/g, 201 km downstream. Sediment samples from the Milltown Reservoir had arsenic concentrations ranging from 6 to 56 μg/g (Brumbaugh et al. 1994). Total recoverable arsenic in nonfiltered pore water from the Clark Fork River decreased from 1,740 μg/L at the farthest upstream sampling station to 31 μg/L at the 201 km station (Brumbaugh et al. 1994). The Coeur d'Alene river basin in northern Idaho has been contaminated with heavy metals from mining and smelting operations since 1885 (Farag et al. 1998). A 1994 study determined the metal content of sediment, biofilm, and invertebrates at 13 sites in the basin, 10 with historic mining activity and 3 reference sites. The mean arsenic levels in sediment at the mining sites ranged from 8.3 to 179.0 μg/g dry weight, compared to 2.4–13.1 μg/g dry weight at the reference sites. The mean arsenic levels in biofilm adhering to rock in the water at the mining sites ranged from 7.5 to 155.8 μg/g dry weight, compared to 7.2–27.3 μg/g dry weight at the reference sites. In Whitewood Creek, South Dakota, where as much as 100 million tons of mining and milling waste derived from gold mining activities were discharged between 1876 and 1977, mean and maximum sediment arsenic concentrations were 1,920 and 11,000 μg/g, respectively (USGS 1987). Uncontaminated sediment had mean arsenic levels of 9.2 μg/g. Arsenic concentrations in surface (0–5 cm) sediments from watersheds in Black Hills, South Dakota, an area impacted by gold mining activities, ranged from 23 to 1,951 μg/g dry weight; concentrations from reference areas ranged from 10 to 58 μg/g dry weight (May et al. 2001). Swan Lake, a sub-bay of Galveston Bay in Texas is a highly industrial area that received runoff from a tin smelter in the 1940s and 1950s. Surface sediments at 17 sites where oysters and mussels were collected ranged from 4.53 to 103 μg/g (Park and Presley 1997). A site in the channel leading from the old smelter had arsenic levels of 568 μg/g. Surface sediment was less contaminated than deeper sediment, indicating less arsenic input recently than in the past as a result of the smelter closing.

It has been suggested that the wood preservative most commonly used in dock pilings and bulkheads, CCA, can be toxic to estuarine organisms. Wendt et al. (1996) measured arsenic in surface sediments and oysters from creeks with high densities of docks and from nearby reference creeks with no docks. The average concentrations in the sediments ranged from 14 to 17 μg/g throughout the study area, which is within the range of natural background levels. Weis et al. (1998) sampled sediments along a 10-m transect from CCA-treated wood bulkheads from four Atlantic coast estuaries. Arsenic concentrations were highest in the fine-grained portion of the sediments near the CCA-treated bulkhead (0–1 m); arsenic concentrations were generally at reference levels at distances >1 m from the bulkheads (Weis et al. 1998).

Soils below and around play structures constructed from CCA-treated wood in the City of Toronto, Canada were sampled and analyzed for inorganic arsenic (Ursitti et al. 2004). A mean arsenic concentration of 2.1 μg/g (range 0.5–10 μg/g) was reported in soil samples taken within 1 m of the CCA-treated wood for all play structures. Soil samples that were collected 10 m from the play structures served as a background had arsenic concentration of 2.4 μg/g (range 0.5–13 μg/g). A mean arsenic concentration of 6.2 μg/g (range 0.5–47.5 μg/g) was reported in soil samples taken below CCA-treated wood for all play structures. Of the 217 play structures in the study, 32 had arsenic concentrations under the play structures that exceeded the Canadian federal soil guidelines with arsenic concentrations ranging from 12.4 to 47.5 μg/g. From this study, the authors concluded that arsenic does not migrate laterally, but does accumulate in soil under elevated platforms constructed from CCA-treated wood (Ursitti et al. 2004).

6.4.4 Other Environmental Media

Low levels of arsenic are commonly found in food; the highest levels are found in seafood, meats, and grains. Typical U.S. dietary levels of arsenic in these foods range from 0.02 mg/kg in grains and cereals to 0.14 mg/kg in meat, fish, and poultry (Gartrell et al. 1986). Shellfish and other marine foods contain the highest arsenic concentrations and are the largest dietary source of arsenic (Gunderson 1995a; Jelinek and Corneliussen 1977; Tao and Bolger 1999). Arsenic levels in various fish and shellfish are presented in Table 6-4. In the U.S. Food and Drug Administration (FDA) Total Diet Study, 1991–1997, seafood contained the highest levels of arsenic, followed by rice/rice cereal, mushrooms, and poultry. Concentrations in canned tuna (in oil), fish sticks, haddock (pan-cooked), and boiled shrimp were 0.609–1.470, 0.380–2.792, 0.510–10.430, and 0.290–2.681 mg/kg, respectively (Tao and Bolger 1999). Typically, arsenic levels in foods in the Total Diet Study, 1991–1996 were low, <0.03 mg/kg; only 63 of the 264 foods contained arsenic above this level. Similar results were reported in the Total Diet Study,

Table 6-4. Levels of Arsenic in Fish and Shellfish—Recent Studies

Sample type	Arsenic concentration[a] (μg/g)	Comments	Reference
Yellowtail flounder		Samples collected from Northwest Atlantic 1993	Hellou et al. 1998
Muscle (n=8)	8–37		
Liver (n=6)	7–60		
Gonad (n=6)	1.2–9.4		
Marine organisms		Belgian fish markets in 1991; inorganic arsenic ranged from 0.003 to 0.2 μg/g	Buchet and Lison 1998
Ray (n=8)	16.4		
Cod (n=8)	4.7		
Plaice (n=8)	19.8		
Sole (n=8)	5.1		
Sea-bream (n=8)	2.4		
Mussell (n=8)	3.5		
Bluefin tuna (*Thunnus thynus*) (n=14)	3.2	Virgin Rocks, Grand Banks of Newfoundland, Canada, 1990	Hellou et al. 1992
Fish		National Contaminant Biomonitoring Program, 1984–1985, 112 stations	Kidwell et al. 1995
Bottom feeding (n=2,020)	0.16±0.23 wet weight		
Predatory (n=12)	0.16±0.140 wet weight		
Oysters		South Carolina, private residential docks on tidal creeks, 1994	Wendt et al. 1996
<1 m from docks (n=10)	8.3±1.1		
>10 m from docks (n=10)	7.6±0.9		
Reference (no docks) (n=10	8.4±1.3		
Clams (n=22)	12±1.1	Indian River Lagoon, Florida, 22 sites, 1990	Trocine and Trefry 1996
Marine organisms		Swan Lake, Galveston Bay, Texas, 1993	Park and Presley 1997
Snails	13.3±17.0		
Blue crab	6.61		
Fish	0.82		
Shrimp	1.37±0.64		
Whole crab	5.35±2.51		
Oysters (n=10, pooled)	7.28±1.32		
Mussels (n=7, pooled)	7.75±2.15		
Marine organisms		GPNEP, 1992, Galveston Bay, Texas	Park and Presley 1997
Blue crab	2.31±2.15		
Fish	2.46		
Oysters, two areas		NOAA NS&T Program, 1986–1990	Park and Presley 1997
n=78, pooled	4.50±1.08	Galveston Bay	
n=874, pooled	9.67±7.00	Gulf of Mexico	

Table 6-4. Levels of Arsenic in Fish and Shellfish—Recent Studies

Sample type	Arsenic concentration[a] (μg/g)	Comments	Reference
Marine crustaceans			
Parapenaeus longirostris (pink shrimp) (n=826, 10 pools	34.84±19.21 (12.01–62.60)	Commercial crustaceans from the Mediterranean Sea (Italy)	Storelli and Marcotrigiano 2001
Aristeus antennatus (red shrimp) (n=387, 8 pool)	17.09±3.49 (10.45–20.82)		
Plesionika martia (shrimp) (n=456, 7 pools)	40.82±2.50 (36.37–44.06)		
Nephrops norvegicus (Norway lobster) (n=270, 5 pools)	43.48±14.21 (35.63–69.15)		
Freshwater fish			
Sabalo (*Brycon melanopterus)* (n=3)	0.015–0.101	Fish samples (muscle) were collected in August 1997 from the Candamo River, Peru; a pristine rainforest valley prior to the start of oil-drilling activities	Gutleb et al. 2002
Huazaco (*Hoplias malabaracus)* (n=4)	nd–0.005		
Bagre (*Pimelodus ornatus*) (n=8)	nd–0.201		
Boquichio (*Prochilodus nigricans)* (n=1)	0.063		
Doncello (*Pseudo-platystoma sp.*) (n=1)	0.055		
Freshwater fish			
Bowfin (n=59)	0.32±0.04 wet weight	Savannah River, along and below the Department of Energy's Savannah River Site (SRS); samples analyzed were edible fillets	Burger et al. 2002
Bass (n=47)	0.03±0 wet weight		
Channel catfish (n=50)	0.09±00.02 wet weight		
Chain pickerel (n=19)	0.05±0.01 wet weight		
Yellow perch (n=51)	0.05±0.01 wet weight		
Black crappie (n=52)	0.04±0.01 wet weight		
American eel (n=24)	0.04±0.01 wet weight		
Shellcracker n=52)	0.06±0 wet weight		
Bluegill (n=52)	0.05±0.02 wet weight		
Redbreast (n=43)	0.07±0.01 wet weight		
Spotted sucker (n=35)	0.03±0 wet weight		

Table 6-4. Levels of Arsenic in Fish and Shellfish—Recent Studies

Sample type	Arsenic concentration[a] (μg/g)	Comments	Reference
Horseshoe crabs			
Apodeme (n=74)	7.034±0.65 wet weight	Overall mean in tissues of crabs collected from New Jersey in 2000	Burger et al. 2003
Egg (n=63)	5.924±0.345 wet weight		
Leg (n=74)	14.482±0.685 wet weight		
Apodeme (n=40)	7.513±0.835 wet weight	Overall mean in tissues of crabs collected from Delaware in 2000	
Egg (n=35)	6.766±0.478 wet weight		
Leg (n=40)	18.102±1.489 wet weight		

[a]Concentrations are means±standard deviation, unless otherwise stated. Concentrations are in a dry weight basis, unless otherwise stated.

GM = geometric mean; GPNEP = Galveston Bay National Estuary Program; nd = not detected;
NOAA NS&T = National Oceanic and Atmospheric Administration National Status and Trends

1991–1997, where the mean arsenic concentration in all foods was 0.036 mg/kg dry weight and arsenic was not detectable in about 88% of the foods and was detected at trace levels in another 7.8% of foods. The foods with the highest mean arsenic levels were haddock, canned tuna, fish sticks, shrimp, and fish sandwiches, with arsenic concentrations ranging from 5.33 to 0.568 mg/kg dry weight (Capar and Cunningham 2000). Nriagu and Lin (1995) analyzed 26 brands of wild rice sold in the United States and found arsenic levels ranging from 0.006 to 0.142 μg/g dry weight. Arsenic concentrations ranging from 0.05 to 0.4 μg/g are typically reported for rice from North America, Europe, and Taiwan (Meharg and Rahman 2003).

During a comprehensive total diet study extending from 1985 to 1988, foods were collected in six Canadian cities and processed into 112 composite food samples (Dabeka et al. 1993). The mean, median, and range of total arsenic in all samples were 0.0732, 0.0051, and <0.0001–4.840 μg/g, respectively. Food groups containing the highest mean arsenic levels were fish (1.662 μg/g), meat and poultry (0.0243 μg/g), bakery goods and cereals (0.0245 μg/g), and fats and oils (0.0190 μg/g). Of the individual samples, marine fish had the highest arsenic levels, with a mean of 3.048 μg/g for the cooked composites and 2.466 μg/g for the raw samples. Canned fish (1.201 μg/g) and shellfish (2.041 μg/g) also contained high means. Cooked poultry, raw mushrooms, and chocolate bars contained 0.100, 0.084, and 0.105 μg/g, respectively.

National monitoring data from the Food Safety and Inspection Service National Residue Program (NRP) (1994–2000) found that the mean total arsenic concentration in livers of young chickens ranged from 0.33 to 0.43 μg/g, with an overall mean of 0.39 μg/g (Lasky et al. 2004). The mean arsenic concentrations in liver for mature chickens, turkeys, hogs, and all other species over the same time period ranged from 0.10 to 0.16 μg/g. Lasky et al. (2004) used the NRP arsenic data in livers of young chickens to estimate the concentrations of arsenic in muscle tissue, the most commonly consumed part of the chicken. Assuming that 65% of the arsenic in poultry and meat is inorganic, at a mean level of chicken consumption of 60 g/person/day, people may ingest an estimated 1.38–5.24 μg/day of inorganic arsenic from chicken.

A Danish study (Pedersen et al. 1994) reports the arsenic levels in beverages as the mean (range) in μg/L as follows: red wine, 9 (<2–25); white wine, 11 (<2–33); fortified wine, 5 (<2–11); beer, 7 (4–11); soft drinks, 3 (<2–8); miscellaneous juices, 8 (3–13); instant coffee, 4 (0.7–7); and instant cocoa, 5.6 (1.6–12.8).

In a study of dietary arsenic exposure in the Indigenous Peoples of the western Northwest Territories, Canada, fish contained the highest arsenic concentrations in foods consumed by the Dene and Métis populations with the highest concentration, 1.960 μg/g, found in smoked/dried cisco (fish). Other foods derived from land mammals, birds, and plants contained lower arsenic concentrations. A mean arsenic intake of <1.0 μg/kg/day was reported for this population (Berti et al. 1998).

The general consensus in the literature is that about 85–>90% of the arsenic in the edible parts of marine fish and shellfish is organic arsenic (e.g., arsenobetaine, arsenochloline, dimethylarsinic acid) and that approximately 10% is inorganic arsenic (EPA 2003b). However, the inorganic arsenic content in seafood may be highly variable. For example, a study in the Netherlands reported that inorganic arsenic comprised 0.1–41% of the total arsenic in seafood (Vaessen and van Ooik 1989). Buchet et al. (1994) found that, on the average, 3% of the total arsenic in mussels was inorganic in form. Some commercially available seaweeds, especially brown algae varieties, may have high percentages of the total arsenic present as inorganic arsenic (>50%) (Almela et al. 2002; Laparra et al. 2003). Arsenic concentrations ranging from 17 to 88 mg/kg dry weight were found in commercially available seaweeds (van Netten et al. 2000). Other arsenic compounds that may be found in seafood are arsenic-containing ribose derivatives called arsenosugars. Arsenosugars are the common organoarsenicals found in marine algae; they are also found in mussels, oysters, and clams (Le et al. 2004). Less information about the forms of arsenic in freshwater fish is known at this time (EPA 2003b).

Schoof et al. (1999a) reported on the analysis of 40 commodities anticipated to account for 90% of dietary inorganic arsenic intake. In this study, the amount of inorganic arsenic was measured in these foods. Consistent with earlier studies, total arsenic concentrations were highest in the seafood sampled (ranging from 160 ng/g in freshwater fish to 2,360 ng/g in marine fish). In contrast, average inorganic arsenic in seafood ranged from <1 to 2 ng/g. The highest inorganic arsenic concentrations were found in raw rice (74 ng/g), followed by flour (11 ng/g), grape juice (9 ng/g), and cooked spinach (6 ng/g).

Tobacco contains an average arsenic concentration of 1.5 ppm, or about 1.5 μg per cigarette (EPA 1998j). Before arsenical pesticides were banned, tobacco contained up to 52 mg As/kg, whereas after the ban, maximum arsenic levels were reduced to 3 μg/g (Kraus et al. 2000). An international literature survey reports arsenic yields of 0–1.4 μg/cigarette for mainstream (inhaled) cigarette smoke (Smith et al. 1997). The wide range of arsenic yields for flue-cured cigarettes suggests that the field history, soil, and fertilizer conditions under which the tobacco is grown will affect the arsenic concentration (Smith et al. 1997).

Arsenic emission factors of 0.015–0.023 μg/cigarette (mean 0.018±0.003 μg/cigarette) have been measured for sidestream smoke from a burning cigarette (Landsberger and Wu 1995).

A median arsenic concentration of 2.1 μg/g and a deposition rate of 0.008 μg/m^2/day was reported in house dust in homes evaluated as part of the German Environmental Survey in 1990–1992. A mean arsenic concentration of 7.3 μg/g was reported in house dust from 48 residences in Ottawa, Canada (Butte and Heinzow 2002). These arsenic concentrations are expected to be representative of background levels. In general high arsenic concentrations were found in household dust collected from homes in areas with known arsenic contamination. Mean arsenic concentrations of 12.6 (2.6–57) and 10.8 (1.0–49) μg/g were reported in house dust collected from the entryway and child play areas, respectively, from homes in a community in Washington State with a history of lead arsenate use (Wolz et al. 2003). Arsenic was detected in all 135 indoor floor dust samples collected as part of the NHEXAS from Arizona mining communities, ranging from 0.3–50.6 μg/g, (O'Rourke et al. 1999). A geometric mean arsenic concentration of 10.8 μg/g (range 1.0–172 μg/g) was reported in house dust from 96 homes in Middleport, New York, with historical pesticide manufacture, collected during the summer and fall of 2003 (Tsuji et al. 2005).

Arsenic has also been detected in several homeopathic medicines at concentrations up to 650 μg/g (Kerr and Saryan 1986). Some Asian proprietary medicines that are manufactured in China, Hong Kong, and other Asian countries have been reported to contain levels of inorganic arsenic ranging from 25 to 107,000 μg/g (Chan 1994). Fifty medicinally important leafy samples that were analyzed for elemental concentrations contained arsenic at levels ranging from 0.12 to 7.36 μg/g, with a mean of 2.38±1.2 μg/g (Reddy and Reddy 1997). Arsenic concentrations ranged from 0.005 to 3.77 μg/g in 95 dietary supplements purchased from retail stores in the Washington, DC area in 1999 (Dolan et al. 2003). Commercially available samples of Valarian, St. John's Wort, Passion Flower, and Echinacea were purchased in the United States and analyzed for various contaminants; arsenic concentrations were 0.0016–0.0085, 0.0065–0.017.8, 0.0024–0.0124, and 0.0021–0.0102 μg/g, respectively, in these samples (Huggett et al. 2001). Concentrations of heavy metals including the metalloid arsenic were evaluated in 54 samples of Asian remedies that were purchased in stores in Vietnam and Hong Kong that would be easily accessible to travelers, as well as in health food and Asian groceries in Florida, New York, and New Jersey. Four remedies were found to contain daily doses exceeding 0.1 mg. Two of these contained what would have been a potentially significant arsenic dose, with daily doses of 16 and 7.4 mg of arsenic (Garvey et al. 2001).

The possible presence of toxic compounds in waste materials has raised concerns about the fate of these compounds either during the composting process or when the composted product is applied to soils. Three waste compost products generated at the Connecticut Agricultural Experiment Station had arsenic levels of 12.8, 9.8, and 13 μg/g dry weight, respectively (Eitzer et al. 1997). The arsenic levels in municipal solid waste composts from 10 facilities across the United States ranged from 0.9 to 15.6 μg/g dry weight with a mean of 6.7 μg/g (He et al. 1995). These are lower than the EPA 503 regulatory limit for arsenic of 41 μg/g for agricultural use of sewage sludge (EPA 1993b). Concentrations of arsenic in U.S. sewage sludges, which are sometimes spread on soil, were <1 μg/g. Arsenic is a common impurity in minerals used in fertilizers. A comprehensive Italian study found that the arsenic content in a number of mineral and synthetic fertilizers ranged from 2.2 to 322 mg/kg with a sample of triple superphosphate having the highest level (Senesi et al. 1999). Arsenic naturally occurs in coal and crude oil at levels of 0.34–130 and 0.0024–1.63 ppm, respectively, which would account for its presence in flue gas, fly ash, and bottom ash from power plants (Pacyna 1987).

Background arsenic levels in living organisms are usually <1 μg/g wet weight (Eisler 1994). Levels are higher in areas with mining and smelting activity or where arsenical pesticides were used. Eisler (1994) has an extensive listing of arsenic levels in terrestrial and aquatic flora and fauna from literature sources to about 1990. The U.S. Fish and Wildlife Service's National Contaminant Biomonitoring Program have analyzed contaminants in fish at 116 stations (rivers and the Great Lakes) across the United States. The geometric mean concentration of arsenic for the five collection periods starting in 1976 were (period, concentration wet weight basis): 1976–1977, 0.199 μg/g; 1978–1979, 0.129 μg/g; 1980–1981, 0.119 μg/g; 1984, 0.106 μg/g; and 1986, 0.083 μg/g (Schmitt et al. 1999). In 1986, the maximum and 85th percentile arsenic levels were 1.53 and 0.24 μg/g, respectively. The highest concentrations of arsenic for all five collection periods were in bloaters from Lake Michigan at Sheboygan, Wisconsin. Arsenic levels declined by 50% at this site between 1976–1997 and 1984. The major source of arsenic into Lake Michigan was a facility at Marinette, Wisconsin, which manufactured arsenic herbicides. Table 6-4 contains arsenic levels in aquatic organisms from more recent studies. The Coeur d'Alene river basin in northern Idaho has been contaminated with heavy metals from mining and smelting operations since 1885 (Farag et al. 1998). A 1994 study determined the metal content of sediment, biofilm, and invertebrates at 13 sites in the basin, 10 with historic mining activity, and 3 reference sites. The mean arsenic levels in benthic macroinvertebrates at the mining sites ranged from 2.2 to 97.0 μg/g dry weight, compared to 2.1–2.4 μg/g dry weight at the reference sites. A study of aquatic organism in Swan Lake, a highly polluted sub-bay of Galveston Bay, Texas showed that arsenic concentrations were in the order snail>oyster>crab>shrimp>fish (Park and Presley 1997). In contrast to metals like silver, cadmium,

copper, and zinc, arsenic concentrations in oysters and mussels were less than in the sediment from which they were collected. No significant correlation was found between levels of arsenic in clams in the Indian River Lagoon in Florida with those found in sediment or water samples (Trocine and Trefry 1996). Small animals living at mining sites ingest more arsenic in their diet and have higher arsenic levels in their bodies than those living on uncontaminated sites (Erry et al. 1999). Seasonal variations in both arsenic intake and dietary composition may affect the amount of arsenic taken up by the body and transferred to predator animals. Tissue arsenic content of wood mice and bank voles living on both arsenic-contaminated mining sites and uncontaminated sites were greater in autumn than spring. The lower tissue arsenic levels in spring of rodents living on contaminated sites suggest that there is no progressive accumulation of arsenic in overwintering animals.

6.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

Exposure to arsenic may include exposure to the more toxic inorganic forms of arsenic, organic forms of arsenic, or both. While many studies do not indicate the forms of arsenic to which people are exposed, this information may often be inferred from the source of exposure (e.g., fish generally contain arsenic as arsenobetaine). Yost et al. (1998) reported that the estimated daily dietary intake of inorganic arsenic for various age groups ranged from 8.3 to 14 μg/day and from 4.8 to 12.7 μg/day in the United States and Canada, respectively, with 21–40% of the total dietary arsenic occurring in inorganic forms.

Drinking water may also be a significant source of arsenic exposure in areas where arsenic is naturally present in groundwater. While estimates of arsenic intake for typical adults drinking 2 L of water per day average about 5 μg/day (EPA 1982c), intake can be much higher (10–100 μg/day) in geographical areas with high levels of arsenic in soil or groundwater (see Figure 6-2). It is assumed that nearly all arsenic in drinking water is inorganic (EPA 2001).

In the United States, food intake of arsenic has been estimated to range from 2 μg/day in infants to 92 μg/day in 60–65-year-old men (see Table 6-5) (Tao and Bolger 1999). The average intake of inorganic arsenic are estimated to range from 1.34 μg/day in infants to 12.54 μg/day in 60–65-year-old men. Tao and Bolger (1999) assumed that 10% of the total arsenic in seafood was inorganic and that 100% of the arsenic in all other foods was inorganic. The greatest dietary contribution to total arsenic was seafood (76–96%) for all age groups, except infants. For infants, seafood and rice products contributed 42 and 31%, respectively. Adult dietary arsenic intakes reported for other countries range

Table 6-5. Mean Daily Dietary Intake of Arsenic for Selected U.S. Population Groups

Mean daily intake (μg/kg body weight/day)	Date of study		
	1984–1986[a]	1986–1991[b]	1991–1997[c]
Provisional tolerable daily intake (PTDI)[d]	2.1	2.1	2.1
6–11 months	0.82	0.5	0.31
2 years	1.22	0.81	1.80
14–16 years, female	0.54	0.36	0.41
14–16 years, male	0.60	0.39	0.24
25–30 years, female	0.66	0.44	0.44
25–30 years, male	0.76	0.51	0.72
60–65 years, female	0.71	0.46	1.08
60–65 years, male	0.74	0.48	1.14

[a]Gunderson 1995a
[b]Gunderson 1995b
[c]Tao and Bolger 1999
[d]No agreement has been reached on a maximum acceptable intake for total arsenic; the FAO/WHO has assigned a PTDI for inorganic arsenic of 2.1 μg/kg body weight for adults. Data from FDA studies. FDA does not recommend daily intake levels for Arsenic.

from 11.7 to 280 μg/day (Tao and Bolger 1999). Schoof et al. (1999b) estimated that intake of inorganic arsenic in the U.S. diet ranges from 1 to 20 μg/day, with a mean of 3.2 μg/day. In contrast, these estimates of inorganic arsenic intakes are based on measured inorganic arsenic concentrations from a market basket survey.

The FDA conducted earlier Total Diet Studies in 1984–1986 and 1986–1991. For the sampling period of June 1984 to April 1986, the total daily intake of arsenic from foods was 58.1 μg for a 25–30-year-old male with seafood contributing 87% of the total (Gunderson 1995a). For the sampling period from July 1986 to April 1991, the total daily intake of arsenic from foods was lower, 38.6 μg for a 25–30-year-old male. Seafood again was the major source of arsenic, contributing 88% of the total (Gunderson 1995b). Results of the two Total Diet Studies for selected population groups are shown in Table 6-5. The Total Diet Study for the sampling period from September 1991 to December 1996, shows that arsenic, at ≥0.03 μg/g, was found in 55 (21%) of the 261–264 foods/mixed dishes analyzed. The highest concentrations again were found in seafood, followed by rice/rice cereal, mushrooms, and poultry. The estimated total daily intake of arsenic from foods was 56.6 μg for a 25–30-year-old male. Seafood was the major contributor, accounting for 88–96% of the estimated total arsenic intake of adults.

Average daily dietary exposures to arsenic were estimated for approximately 120,000 U.S. adults by combining data on annual diet, as measured by a food frequency questionnaire, with residue data for table-ready foods that were collected for the annual FDA Total Diet Study. Dietary exposures to arsenic were highly variable, with a mean of 50.6 μg/day (range, 1.01–1,081 μg/day) for females and 58.5 μg/day (range, 0.21–1,276 μg/day) for males (MacIntosh et al. 1997). Inorganic arsenic intake in 969 men and women was assessed by a semi-quantitative food frequency questionnaire in combination with a database for total arsenic content in foods and by toenail concentrations of arsenic. The mean estimated average daily consumption of inorganic arsenic was 10.22 μg/day with a range of 0.93–104.89 μg/day. An assumption of 1.5% of the total arsenic in fish and 20% of the total arsenic in shellfish was inorganic arsenic was used in this assessment (MacIntosh et al. 1997).

During a comprehensive total diet study extending from 1985 to 1988, the estimated daily dietary ingestion of total arsenic by the average Canadian was 38.1 μg and varied from 14.9 μg for the 1–4 year-old-age group to 59.2 μg for 20–39-year-old males (Dabeka et al. 1993). Daily intakes of arsenic from food by women in the Shiga Prefecture, Japan, were investigated by the duplicate portion method and by the market basket method. In 1991 and 1992, the daily intakes determined by the duplicate portion

method were 206 and 210 μg, respectively. Those determined by the market basket method were 160 and 280 μg, respectively (Tsuda et al. 1995b).

Arsenic concentrations in human breast milk have been reported to range from 4 to <10 μg/L in pooled human milk samples from Scotland and Finland to 200 μg/L in samples from Antofagasta, Chile, where there is a high natural environmental concentration of arsenic (Broomhall and Kovar 1986). The arsenic concentration in the breast milk of 35 women in Ismir, Turkey, a volcanic area with high thermal activity ranged from 3.24 to 5.41 μg/L, with a median of 4.22 μg/L (Ulman et al. 1998). Sternowsky et al. (2002) analyzed breast milk from 36 women from three different regions in Germany. These regions included the city of Hamburg, a rural area, Soltau, Lower Saxony, and Munster, the potentially contaminated area. Arsenic was not detected (<0.3 μg/L) in 154 of 187 samples, with the highest concentration, 2.8 μg/L, found in a sample from the rural area. The geometric means from the three areas were comparable.

The mean arsenic levels in three groups of cows in the region that grazed on land impacted by lava and thermal activity were 4.71, 4.46, and 4.93 μg/L, compared to 5.25 μg/L for cows kept in sheds and fed commercial pellet feed and municipal water (Ulman et al. 1998). Mean arsenic concentrations in cow's milk ranging from 18.6 to 17.1 μg/L and from 16.7 to 18.0 μg/L were reported for cow's grazing in nonindustrial and an industrial regions, respectively, in Turkey (Erdogan et al. 2004).

A Danish study found that carrots grown in soil containing 30 μg/g of arsenic, which is somewhat above the 20 μg/g limit for total arsenic set by Denmark for growing produce, contained 0.014 μg/g fresh weight of arsenic, all in the form of inorganic As(III) and As(V) (Helgesen and Larsen 1998). An adult consuming 376 grams of vegetables a day (90th percentile) represented solely by carrots would consume 5.3 μg of arsenic a day. The study concluded that the estimated intake of arsenic from produce grown in soil meeting regulatory limits was low compared with other food sources and water.

If vegetables are grown in planters made of wood treated with CCA, arsenic may leach out of the wood and be taken up by the vegetables. In a study by Rahman et al. (2004), arsenic was found to diffuse into the soil from the CCA-treated wood, with the highest concentrations found at 0–2 cm from the CCA-treated wood and a steady decline in concentration with increased distance from the wood. Crops grown within 0–2 cm of the CCA-treated wood contained higher concentrations of arsenic than those grown at 1.5 m from the treated wood. However, the concentrations are below U.S. FDA tolerance limits that have been set for arsenic in select food items. In addition, food grown in this manner is unlikely to constitute a significant part of a person’s diet.

In 2003, U.S. manufacturers of arsenical wood preservatives began a voluntary transition from CCA to other wood preservatives in wood products for certain residential uses, such as play structures, picnic tables, decks, fencing, and boardwalks. This phase out was completed on December 31, 2003; wood treated prior to this date could still be used and structures made with CCA-treated wood would not be affected. CCA-treated wood products continue to be used in industrial applications (EPA 2003a). EPA's Consumer Awareness Program (CAP) for CCA is a voluntary program established by the manufacturers of CCA products to inform consumers about the proper handling, use, and disposal of CCA-treated wood. Additional information about this program can be found from EPA (2007a).

The arsenic content in the human body is 3–4 mg and tends to increase with age. Arsenic concentrations in most tissues of the human body are <0.3 to 147 μg/g dry weight, excluding hair, nails, and teeth. Mammals tend to accumulate arsenic in keratin-rich tissues such as hair and nails. The normal concentrations of arsenic range from about 0.08 to 0.25 μg/g in hair, and 0.34 μg/g in nails. The normal concentration of arsenic in urine can range from 5 to 40 μg per day (total) (Mandal and Suzuki 2002). Table 6-6 contains arsenic levels in various human tissues.

A German study investigated the transfer of arsenic from the environment to humans in the northern Palatine region, a former mining area characterized by high soil levels of arsenic (<2–605 μg/g) in residential areas compared to a region in southern lower Saxony with nonelevated levels of arsenic in soil (Gebel et al. 1998a). None of the residents were occupationally exposed to arsenic and the arsenic levels in drinking water were generally below 0.015 mg/L. The mean levels of arsenic in urine and hair were lower in the reference area than in the former mining area (see Table 6-6), although within the mining area, there was a slight increase in arsenic levels in hair and arsenic excreted in urine with increasing arsenic content in soil. Children in the Palatine region did not have higher contents of arsenic in their hair or urine. The most significant factor contributing to elevated levels of arsenic in hair and urine was seafood consumption. In the combined population of people living in mining areas containing high levels of arsenic in soil and other areas, the level of arsenic in urine was positively associated with the extent of seafood consumption. However, the study also showed that seafood consumption does not lead to an extreme increase in excretion of arsenic in the urine. There are apparently other, unidentified factors affecting the urine levels. Only arsenic in urine, not in hair, was significantly correlated with age. The level of arsenic in urine was very slightly, but significantly correlated with the consumption of home-grown produce. Tobacco smoking had no correlation with the arsenic content of either hair or urine (Gebel et al. 1998a).

Table 6-6. Levels of Arsenic in Human Tissue and Urine—Recent Studies

Site population	Sample	Concentration Mean[a]	Range	Units	Reference
Fort Valley, Georgia, Pesticide manufacturing facility (Superfund site)					
40 workers (samples collected at end of work week)	Urine, random	11.6	<1–57	μg/L	Hewitt et al. 1995
	Urine, 24-hour	11.0	<1–54	μg/L	
	Hair	0.78	<0.01–6.3	μg/g	
	Fingernails	0.79	<0.01–6.1	μg/g	
Hermosa, Sonora, Mexico					
Children, ages 7–11, exposed to arsenic in water (mean concentration [mean dose]):	Urine, 24-hour				Wyatt et al. 1998a, 1998b
9 μg/L [0.481 μg/kg/day]		10.26	4.05–19.68	μg/day	
15 μg/L [0.867 μg/kg/day]		10.54	2.82–20.44	μg/day	
30 μg/L [1.92 μg/kg/day]		25.18	5.44–93.28	μg/day	
Glasgow, Scotland					
Adults, normal (n=1,250)	Hair	0.650	0.20–8.17	μg/g	Raie 1996
Adults, postmortem (n=9)	Liver	0.048 [0.024]	0.011–0.152	μg/g	
Infants, postmortem (n=9)	Liver	0.0099 [0.007]	0.0034–0.019	μg/g	
Adults, postmortem (n=8)	Lung	0.044 [0.022]	0.0121–0.125	μg/g	
Infants, postmortem (n=9)	Lung	0.007 [0.0055]	0.0011–0.015	μg/g	
Adults, postmortem (n=9)	Spleen	0.015 [0.008]	0.001–0.063	μg/g	
Infants, postmortem (n=8)	Spleen	0.0049 [0.0045]	0.0011–0.0088	μg/g	
Palatinate Region, Germany (high As)[b]					
Residents (n=199)	Urine, 24-hour	3.96 [3.21]	<0.1–18.32	μg/g	Gebel et al. 1998a
Residents (n=211)	Hair	0.028 [0.016]	<0.005–0.154	μg/g	
Saxony, Germany (low As—reference)[b]					
Residents (n=75)	Urine, 24-hour	7.58 [6.20]	0.29–23.78	μg/g	Gebel et al. 1998a
Residents (n=74)	Hair	0.069 [0.053]	0.013–0.682	μg/g	
Ismir, Turkey, (volcanic area with high thermal activity)					
Nonoccupationally exposed women (n=35)	Breast milk	4.23 [4.26]	3.24–5.41	μg/L	Ulman et al. 1998
Erlangen-Nurenberg Germany 1/92–12/93					
Nonoccupationally exposed people (n=50)	Lung	5.5	<1–13.0	ng/g ww	Kraus et al. 2000
		28.4	<1–73.6	ng/g dw	

Table 6-6. Levels of Arsenic in Human Tissue and Urine—Recent Studies

Site population	Sample	Concentration		Units	Reference
		Mean[a]	Range		
Tarragona (Catalonia, Spain) 1997–1999					
Nonoccupationally exposed people (n=78)	Lung	<0.05		µg/g ww	Garcia et al. 2001
	Bone	<0.05			
	Kidney	<0.05			
	Liver	<0.05			
	Lung	<0.05			
West Bengal, India					
Residents consuming arsenic-contaminated water (n=47)	Fingernail	7.32	2.14–40.25	µg/g	Mandal et al. 2003
	Hair	4.46	0.70–16.17		
Residents consuming nonarsenic-contaminated water (n=15)	Fingernail	0.19	0.11–0.30		
	Hair	0.07	0.03–0.12		
Middleport, NY, USA					
Children <7 years (n=77)	Urine	15.1[c]	2.1–59.6	µg/L	Tsuji et al. 2005
Children <13 years (n=142)	Urine	15.7[c]	2.1–59.9		
Children ≥7 years and adults (n=362)	Urine	15.8[c]	3.9–773		
All participants	Urine	15.7[c]	2.1–773		

[a]Medians, if reported, are in brackets.
[b]The reference group (Saxony) had significantly higher levels of arsenic in urine and hair. However, data from both groups correspond to normal range reference data.
[c]Geometric mean, total arsenic

dw = dry weight; ww = wet weight

A study was performed to look at the arsenic levels, as well as the arsenic species present, in hair and nail samples from individuals in an arsenic-affected area in West Bengal, India. Mean arsenic concentrations in hair and fingernails of the chronically arsenic exposed population were 4.46 and 7.32 μg/g, respectively and were 0.07 and 0.19 μg/g in a control population. Fingernail samples were found to contain mostly inorganic arsenic (>80%) as a mixture of As(III) and As(V), as well as DMA(III) and DMA(V). Hair samples also mostly contained inorganic arsenic (>90%), as well as MMA(V) and DMA(V) (Mandal et al. 2003).

Arsenic in soil in communities surrounding former smelters is a public health concern, especially for infants and children who may consume significant quantities of soil. Since lead arsenate was used in apple and other fruit orchards, often at very high application rates, and this compound would be expected to accumulate and persist in surface soil, there are concerns to human health when these when old orchards are converted into subdivisions or when they are used to grow food crops or forage. However, arsenic in soil may be imbedded in minerals or occur as insoluble compounds such as sulfides and therefore, not be taken up by the body from the gastrointestinal tract. In addition, oxidation of mineral surfaces may result in armoring the primary mineral grain by a secondary reaction product. Arsenic-bearing solids are often encapsulated in insoluble matrices such as silica, further diminishing arsenic availability (Davis et al. 1992).

Sarkar and Datta (2004) examined the bioavailability of arsenic from two soils with different arsenic retention capacities. In this study, Immokalee (Florida) and Orelia (Texas) soils were incubated after spiking with sodium arsenate for 4 months. The Immokalee soil is a sandy spodosol with low Fe/Al, Ca/Mg, and P contents and is likely to have minimal arsenic retention capacity. The Orelia soil is a sandy clay that is expected to have strong arsenic retention capacity. Arsenic speciation and bioavailability were studied immediately after spiking and after 4 months of incubation. Approximately 85% of the total arsenic (soluble and exchangeable fractions) was considered bioavailable and phytoavailable immediately after pesticide application for the Immokalee soil; after 4 months of incubation, this decreased to approximately 46%. Immediately after pesticide application, the amounts of arsenic extracted in the soluble/exchangeable and Fe/Al-bound fractions were similar that of the Immokalee soil. After 4 months, the soluble arsenic decreased to approximately 45% and the Fe/Al-bound arsenic increased to about 40%. Experiments looking at the bioavailability of arsenic from these two soils indicated that the potentially irreversible adsorption of arsenic by the Orelia soil rendered a significant portion of the total arsenic unavailable for absorption by the human gastrointestinal system. Initially after pesticide application,

100% of the arsenic was bioavailable; after 4 months, the bioavailable fraction was found to decrease to 88 and 69% in the Immokalee and Orelia soils, respectively (Sarkar and Datta 2004).

Hamel et al. (1998) used synthetic gastric juice to estimate the bioaccessible fraction of metals in the stomach with varying liquid to solid ratios. They found that the bioaccessibility may vary in different soils and with varying liquid to solid ratios. Bioaccessibility was defined as the amount of metal that is soluble in synthetic gastric juice and therefore, potentially available for uptake across the intestinal lumen, while bioavailability was defined as the amount that was actually taken across the cell membranes. Arsenic bioaccessibility for National Institute of Standards and Technology (NIST) Montana Soil SRM 2710, with a certified arsenic concentration of 626 μg/g, was fairly consistent across the liquid-to-solid ratios and ranged from 41.8±18 to 56±21%. The extractability of a hazardous waste contaminated soil from Jersey City, New Jersey, was different than that observed for the Montana NIST soil. For the Jersey City soil, which had an arsenic concentration of 1,120 μg/g, there was an increase in the bioaccessible arsenic as the liquid-to-solid ratio increased. Bioaccessible arsenic ranged from 4.5±0.8 (at a liquid-to-solid ratio of 100:1) to 25±9% (at a ratio of 5,000:1). Similarly, smelter impacted soils from Anaconda, Montana contain metal-arsenic oxides and phosphates whose bioaccessibility is limited by solubility restraints for residence times typical of the gastrointestinal tract (Davis et al. 1992, 1996).

Inhalation of arsenic from ambient air is usually a minor exposure route for the general population. For example, the dose to a person who breathes 20 m^3/day of air containing 20–30 ng/m^3 (see Section 6.4.1) would be about 0.4–0.6 μg/day. However, smokers may be exposed to arsenic by inhalation of mainstream smoke. Assuming that 20% of the arsenic in cigarettes is present in smoke, an individual smoking two packs of cigarettes per day would inhale about 12 μg of arsenic (EPA 1984a). However, a German study of the arsenic levels in lung tissue of 50 unexposed deceased people (see Table 6-6) found no significant difference in lung arsenic concentrations of smokers versus nonsmokers, nor were there any significant age- or sex-related differences (Kraus et al. 2000). Before arsenical pesticides were banned, tobacco contained up to 52 μg As/g, whereas after the ban, maximum arsenic levels were reduced to 3 μg/g.

Occupational exposure to arsenic may be significant in several industries, mainly nonferrous smelting, arsenic production, wood preservation, glass manufacturing, and arsenical pesticide production and application. Since arsenic compounds are used as a desiccant for cotton, workers involved in harvesting and ginning cotton may be exposed to arsenic. Occupational exposure would be via inhalation and dermal contact. Should any arsenic be retained in the cotton, workers handling the fabric and the general

public would be exposed. The electronics industry is expanding the use of gallium arsenide in the production of electro-optical devices and integrated circuits, and workers in the industry where gallium arsenide is used may be exposed to hazardous substances such as arsenic, arsine, and various acids (Sheehy and Jones 1993). Occupational exposure to arsenic is generally assessed by measuring urinary excretion of arsenic. Past exposure is commonly assessed by arsenic levels in hair. Different types of occupational exposures may result in different uptakes of arsenic because of the bioavailability of the form of arsenic to which workers are exposed. For example, maintenance workers at a Slovak coal-fired power plant exposed to 8-hour TWA arsenic air concentrations of 48.3 μg/m^3 (range, 0.17–375.2) had urinary total arsenic levels of 16.9 μg As/g creatinine (range, 2.6–50.8), suggesting that bioavailability of arsenic from airborne coal fly ash is about one-third that from in copper smelters and similar settings (Yager et al. 1997). Approximately 90% of the arsenic-containing particulates were ≥3.5 μm. Apostoli et al. (1999) monitored 51 glass workers exposed to arsenic trioxide by measuring dust in the breathing zone. The mean concentration of arsenic in air was 82.9 μg/m^3 (1.5–312 μg/m^3); exposure was higher for workers involved in handling the particulate matter. The occupation exposures to principal contaminants, including arsenic, at five coal-fired power plants were evaluated during June–August 2002. Eight air samples were collected per similar exposure group at four of the five facilities; inorganic arsenic concentrations in all samples were below the limit of detection (0.37–0.72 μg/m^3), as well as being below the OSHA permissible exposure limit (PEL) of 10 μg/m^3 (Bird et al. 2004).

NIOSH researchers conducted a study of arsenic exposures and control systems for gallium arsenide operations at three microelectronics facilities during 1986–1987 (Sheehy and Jones 1993). Results at one plant showed that in all processes evaluated but one, the average arsenic exposures were at or above the OSHA action level of 5 μg/m^3, with a maximum exposure of 8.2 μg/m^3. While cleaning the Liquid Encapsulated Czochralski (LEC) pullers, the average potential arsenic exposure of the cleaning operators was 100 times the OSHA PEL of 10 μg/m^3. Area arsenic samples collected at the plant in break-rooms and offices, 20–60 feet from the process rooms, had average arsenic concentrations of 1.4 μg/m^3. At the other two plants, personal exposures to arsenic were well controlled for all processes evaluated.

A study has been conducted to examine the relationship between total arsenic levels in hair of employees in a semiconductor fabrication facility and job responsibility, a surrogate variable for arsenic exposure (de Peyster and Silvers 1995). Airborne arsenic was found in areas where equipment was cleaned but not in administrative areas. The highest airborne arsenic level found in the study, 15 μg/m^3, was collected from the breathing zone of a maintenance employee who was cleaning a source housing over a period of 2 hours in an area with local exhaust ventilation. A concentration of 2 μg/m^3 was found during the

remainder of the cleaning period (~53 minutes). Workers in maintenance who were regularly assigned to cleaning equipment, and therefore presumed to have the highest exposure potential, had a mean hair arsenic level of 0.042 μg/g. This was higher than the mean of 0.033 μg/g observed in administrative controls, but the difference was not significant. Maintenance workers who only occasionally cleaned and maintained arsenic-contaminated equipment had a mean hair arsenic level of 0.034 μg/g, which was comparable to the controls. The highest group mean hair arsenic level of 0.044 μg/g, surprisingly, was found in supervisors and engineers who were presumed to have the lowest exposure potential of all workers in the process areas. However, the highest concentrations of hair arsenic in engineers, 0.076 and 0.106 μg/g, were observed in two heavy smokers who smoked 1–2 packs of cigarettes per day. A 2-way analysis of variance indicated that smoking appeared to be a significant contributing factor whereas occupational exposure was not.

Hwang and Chen (2000) evaluated arsenic exposure in 21 maintenance engineers (exposed group) and 10 computer programmers (control group) at 3 semiconductor manufacturing facilities. Samples of air, wipe, and urine, as well as used cleaning cloths and gloves were collected to determine arsenic exposure. Arsenic was undetectable in 46 of the 93 air samples, and most samples were generally below the recommended occupational exposure limit (10 $\mu g/m^3$) in work areas during ion implanter maintenance. Arsine was detectable in 22 of the 45 area air samples and in 15 of the 35 personal air samples; however, all concentrations were well below the occupational exposure limit of 50 ppb (160 $\mu g/m^3$). Mean arsine concentrations ranged from not detected to 4.0 ppb (15 $\mu g/m^3$) in area air samples, and the mean arsine concentration of personal air for maintenance engineers was 4.3 ppb (14 $\mu g/m^3$). Arsenic concentrations in wipe samples, used cleaning cloths, and gloves, varied from not detected to 146 $\mu g/cm^2$. During ion implanter maintenance, urinary arsenic levels were found to increase (1.0–7.8 μg/g creatinine) in the maintenance engineers, from a mean baseline concentration of 3.6 μg/g creatinine. The average urinary arsenic level for the computer programmers was 3.8 μg/g creatinine (Hwang and Chen 2000). Mean arsenic concentrations in blood of 103 workers in the optoelectronic industry and 67 controls were 8.58 and 7.85 μg/L, respectively (Liao et al. 2004).

Concentrations of various metals, including arsenic, were measured in autopsy tissues (liver, lung, kidney, brain, and bone) collected from 78 nonoccupationally exposed subjects from Tarragona County, Spain between 1997 and 1999. In general, arsenic concentrations were under the analytical detection limit (0.05 μg/g wet weight) in all tissues (Garcia et al. 2001).

CCA preservatives are commonly used for treating timber used in constructions in marine and other humid environments or in contact with the ground. Exposure to CCA compounds may occur through dermal contact and inhalation of dust while working with the treated timber. Nygren et al. (1992) investigated the occupational exposure to airborne dust, chromium, copper, and arsenic in six joinery shops in Sweden where impregnated wood was used for most of their production. The mean airborne concentration of arsenic around various types of joinery machines ranged from 0.54 to 3.1 $\mu g/m^3$. No increased concentrations of arsenic were found in the workers' urine. A study was carried out in Denmark to evaluate arsenic exposure in taxidermists, workers impregnating wood with CCA solutions, fence builders, construction workers, and workers impregnating electric pylons with arsenic solution (Jensen and Olsen 1995). Airborne arsenic exposure was documented in 19 of 27 individuals working with products containing arsenic. The maximum exposure concentration was 17.3 $\mu g/m^3$, found for a single worker who was filling an impregnation container with CCA paste. Median exposures for indoor workers producing garden fences and weekend cottages were 3.7 and 0.9 $\mu g/m^3$, respectively. The maximum urine concentration reported in the study was 294.5 nanomoles arsenic per millimole creatinine (195 μg As/g creatinine) and was from the injector impregnating electric pylons. The median concentration in workers on electric pylons was 80 nanomoles arsenic per millimole creatinine (53 μg As/g creatinine), which was 6 times the concentration in reference individuals. Urine arsenic levels in workers producing garden fences and in taxidermists were 2.9 and 1.8 times the reference level, respectively.

The NIOSH National Occupational Exposure Survey (NOES) conducted in 1981–1983 estimated that about 55,000 workers were potentially exposed to arsenic (NOES 1990). The NOES was based on field surveys of 4,490 facilities that included virtually all workplace environments, except mining and agriculture, where eight or more persons are employed. The principal exposure pathway is probably inhalation of arsenic adsorbed to particulates, but ingestion and possibly dermal exposure may also be common. Since arsenic is no longer produced in the United States (see Section 5.1) and many arsenical pesticide uses have been banned (see Chapter 8), it is likely that the number of workers occupationally exposed to arsenic has decreased markedly in more recent years.

6.6 EXPOSURES OF CHILDREN

This section focuses on exposures from conception to maturity at 18 years in humans. Differences from adults in susceptibility to hazardous substances are discussed in Section 3.7, Children's Susceptibility.

Children are not small adults. A child's exposure may differ from an adult's exposure in many ways. Children drink more fluids, eat more food, breathe more air per kilogram of body weight, and have a larger skin surface in proportion to their body volume. A child's diet often differs from that of adults. The developing human's source of nutrition changes with age: from placental nourishment to breast milk or formula to the diet of older children who eat more of certain types of foods than adults. A child's behavior and lifestyle also influence exposure. Children crawl on the floor, put things in their mouths, sometimes eat inappropriate things (such as dirt or paint chips), and spend more time outdoors. Children also are closer to the ground, and they do not use the judgment of adults to avoid hazards (NRC 1993).

As with adults, most children are exposed to arsenic largely through their diet. Since the greatest dietary intake of arsenic is from fish and seafood, infants and young children for whom a substantial part of their food is milk, would not be exposed to arsenic from dietary sources as much as older children. Even when mothers consumer large amounts of seafood, there does not appear to be any major transfer of arsenobetaine, the major form of arsenic in seafood, from seafood to milk (Grandjean et al. 1995). Arsenic concentrations were very low in human milk sampled from 88 mothers in the Faroe Islands, where the seafood diet includes pilot whale meat and blubber. The total arsenic concentrations ranged from 0.1 to 4.4 μg/kg, with a median of 1.6 μg/kg (Grandjean et al. 1995). The arsenic concentration in the breast milk of 35 women in Ismir, Turkey, a volcanic area with high thermal activity ranged from 3.24 to 5.41 μg/L, with a median of 4.22 μg/L (Ulman et al. 1998). The mean arsenic levels in three groups of cows in the region that grazed on land impacted by lava and thermal activity were 4.71, 4.46, and 4.93 μg/L, compared to 5.25 μg/L for cows kept in sheds and fed commercial pellet feed and municipal water. The arsenic levels in the urine of pregnant women and the cord blood of their infants were 0.625±0.027 and 0.825±0.079 μg/L, respectively. The authors concluded that there was no harmful exposure to arsenic in volcanic areas with high arsenic levels from suckling infants or feeding them local cow's milk, nor was there harm to the newborns from their mother's diet. Sternowsky et al. (2002) analyzed breast milk from 36 women from three different regions in Germany. These regions included the city of Hamburg, a rural area, Soltau, Lower Saxony, and Munster, the potentially contaminated area. Arsenic was not detected (<0.3 μg/L) in 154 of 187 samples, with the highest concentration, 2.8 μg/L, found in a sample from the rural area. The geometric mean arsenic concentrations from the three areas were comparable. Calculated oral intakes of arsenic were between 0.12 and 0.37 μg/day for an infant at 3 months of age and weighing 6 kg.

According to the FDA study of 1986–1991, the mean daily intakes of arsenic are 0.5 and 0.81 μg/kg body weight per day for a 6–11-month-old infant and 2-year-old child, respectively (Gunderson 1995b). This

can be compared to a mean daily intake of 0.51 μg/kg-body weight per day for a 25–30-year-old male (see Table 6-5). A Total Diet Study, from September 1991 to December 1996, estimated that the average inorganic arsenic intake for children of various age/sex groups were (age-sex group, total arsenic intake in μg/day, inorganic arsenic intake in μg/day): 6–11 months, 2.15, 1.35; 2 years, 23.4, 4.41; 6 years, 30.3, 4.64; 10 years, 13.3, 4.21; and 14–16 years (females), 21.8, 5.15; 14–16 years (males), 15.4, 4.51 (Tao and Bolger 1999). The greatest dietary contribution (76–96%) of total arsenic intake for all age groups other than infants was seafood. For infants, 41 and 34% of the estimated total arsenic intakes are from seafood and rice/rice cereals, respectively (Tao and Bolger 1999). Only for toddlers does the intake approach the World Health Organization's (WHO) provisional tolerable daily intake (PTDI) for inorganic arsenic (see Table 6-5). A 1985–1988 Canadian total diet study estimated that 1–4-year-olds ingested 14.9 μg of total arsenic per day compared with 38.1 μg by the average Canadian and 59.2 μg for 20–39-year-old males (Dabeka et al. 1993). Yost et al. (2004) estimated the mean dietary intake for inorganic arsenic for children (1–6 years of age) to be 3.2 μg/day, with a range of 1.6–6.2 μg/day for the 10th and 95th percentiles, respectively. Inorganic arsenic intake was predominantly contributed by grain and grain products, fruits and fruit juices, rice and rice products, and milk (Yost et al. 2004). Total arsenic and arsenobentaine concentrations were measured in 16 baby food samples obtained from manufactures in Spain; total arsenic concentrations ranged from 2.042 to 0.270 μg/g in plaice with vegetables and sole with white sauce, respectively. Arsenobetaine, which is the arsenical commonly found in fish, accounted for essentially 100% of the arsenic present in the samples (Vinas et al. 2003).

Arsenic exposure from drinking water may be elevated especially in groundwater from areas where arsenic occurs naturally in soil such as the western and north central sections of the United States (see Table 6-3 and Figure 6-2).

Arsenic exposure in communities near mining and smelting facilities or where arsenic had formerly been applied to agricultural land are a public health concern, especially for infants and children. Since arsenic remains in the surface soil indefinitely and long past land uses may be forgotten, people may not realize that they are living in areas where high levels of arsenic may occur in soil. Contaminated soils pose a particular hazard to children because of both hand-to-mouth behavior and intentional ingestion of soil (pica) that contains metals and other contaminants (Hamel et al. 1998). In these communities, arsenic may contaminate carpeting or may have been tracked in from outside. Children may be exposed to this arsenic while crawling around or playing on contaminated carpeting. Exposure may also result from dermal contact with the soil, or by inhaling the dust and then swallowing it after mucociliary transport up

out of the lungs. Because much of the arsenic in soil is embedded in or adsorbed to soil particles or insoluble, it may not be in a form accessible for uptake by the body.

Hwang et al. (1997b) studied the arsenic exposure of children in Anaconda, Montana, in the vicinity of a former copper smelter from the summer of 1992 through the summer of 1993. Environmental samples and first morning voided urine samples from 414 children <72 months old were collected. Attention was focused on that fraction of the environmental source that was thought to be of the greatest risk to the child (i.e., arsenic in small particles [<250 μm]) that could most readily adhere to hands and toys and could be inadvertently ingested. Average arsenic levels in different types of soil ranged from 121 to 236 μg/g. Several studies have reported mean soil ingestion values for children ranging from 9 to 1,834 μg /day. Assuming that high arsenic exposure areas have average arsenic levels in soil from 60 to 150 μg/g, the resulting daily arsenic intake from soil could range from 1 to 275 μg/day per child. The geometric mean of speciated urinary arsenic (combined As(III), As(V), MMA and DMA) was 8.6±1.7 μg/L (n=289) in the Hwang study. A nationwide survey on arsenic exposure in the vicinity of smelter sites revealed that children without excess arsenic exposure had average total urinary arsenic levels ranging from 5 to 10 μg/L (Hwang et al. 1997a). Compared to these values, the mean total urinary arsenic values found in the Hwang study were markedly higher, but they were still well below the WHO-recommended maximum excretion level for total arsenic of 100 μg/L as an action level for intervention. The investigators hypothesized that the relatively low urinary arsenic levels found in the study were probably a reflection of the low bioavailability of some forms of arsenic in contaminated soil. Hwang et al. (1997a) stated that arsenic intake through skin contact is insignificant and may be neglected in the assessment of childhood arsenic exposure. They recommend that parents or guardians pay more attention to their children's activity, especially hand-to-mouth behavior, even though the environmental contaminants might be elevated only slightly. Children in the northern Palatine region of German study, a former mining area characterized by high levels of arsenic (<2–605 μg/g) in residential areas did not show higher arsenic levels in their hair or urine than children from a reference area of Germany (Gebel et al. 1998a).

While CCA registrants voluntarily canceled the production of CCA-treated wood for residential use in 2003, there is a potential for exposure to arsenic from existing structures (Zartarian et al. 2006). Based on a review of existing studies, Hemond and Solo-Gabriele (2004) estimated that children with contact with CCA-treated wood may be subjected to doses in the range of tens of micrograms of arsenic per day. The most important route of exposure appeared to be by hand-to-mouth activities after contact with the CCA-treated wood. Kwon et al. (2004) compared the amounts of water-soluble arsenic on hands of children in

contact with CCA-treated wood structures or sand in playgrounds. The mean amount of water-soluble arsenic on children's hands from playgrounds without CCA-treated wood was 0.095 μg (range 0.011–0.41 μg). A mean amount of water-soluble arsenic on children's hands from playgrounds with CCA-treated wood was 0.5 μg (range 0.0078–3.5 μg) (Kwon et al. 2004). Additional data from the study by Kwon et al. (2004) showed that total arsenic collected in hand-washing water (insoluble arsenic on the filter combined with the water-soluble arsenic in the filtrate) was 0.934 and 0.265 μg for the CCA playgrounds and the non-CCA playgrounds, respectively (Wang et al. 2005). Two wood surface swab samples collected from 217 play structures constructed from CCA-treated wood in the City of Toronto, Canada were sampled and analyzed for inorganic arsenic (Ursitti et al. 2004). Dislodgeable arsenic concentrations were found to vary widely from nondetectable (0.08–0.25 μg/100 cm^2) to 521 μg/100 cm^2 (mean = 41.6 μg/100 cm^2), and were found to not be a useful predictor of soil arsenic levels (Ursitti et al. 2004).

Shalat et al. (2006) evaluated postexposure hand rinses and urine for total arsenic for 11 children (13–71 months) in homes in Miami-Dade County, Florida, with and without CCA-treated playgrounds. Seven playgrounds were included in this study, and five of these contained either CCA-treated or partially CCA-treated wood. In addition, samples of wood, soil (5–8 cm from the base of the playground structure), mulch (when present), and synthetic wipes were analyzed for total arsenic. Wood and soil arsenic concentration were <2.0 and <3 mg/kg for the non-CCA-treated playgrounds, respectively. Mean arsenic concentrations of 2,380 mg/kg (range 1,440–3,270 mg/kg) and 19 mg/kg (4.0–42 mg/kg) were reported for wood and soil, respectively, in the playgrounds with CCA-treated wood. An arsenic concentration in mulch at one playground without CCA-treated wood was 0.4 mg/kg, and arsenic concentrations were 0.6 and 69 mg/kg in mulch at two of the playgrounds with CCA-treated wood. The amount of arsenic removed by synthetic wipes from the non-CCA-treated wood was <0.5 μg, while the mean amount of arsenic removed from the CCA-treated wood was 117 μg (range 1.0–313 μg). The amount of arsenic in hand rinses from children who played at the playgrounds with non-CCA-treated wood and at the playgrounds with CCA-treated wood were <0.2 and 0.6 μg (range <0.2–1.9 μg), respectively. The mean urinary total arsenic concentration was 0.0136 μg/L (range 0.0072–0.0231 μg/L) for all children. No association between assess to CCA-treated playgrounds and urinary arsenic levels was found (Shalat et al. 2006).

The potential exposure children may receive from playing in play structures constructed from CCA-treated wood is generally smaller than that they would receive from food and water. For comparison, Yost et al. (2004) estimated the mean dietary intake for inorganic arsenic for children (1–6 years of age)

to be 3.2 μg/day, with a range of 1.6–6.2 μg/day for the 10th and 95th percentiles, respectively. In a Total Diet Study, from September 1991 to December 1996, estimated average total intakes for children aged 6–11 months, 2 years, 6 years, and 10 years were 2.15, 23.4, 30.3, and 13.3 μg/day, respectively. Average inorganic arsenic intakes for the same age groups were estimated as 1.35, 4.41, 4.64, and 4.21 μg/day, respectively, based on data for total arsenic in foods and the assumption that 10% of the total arsenic in seafood was inorganic and that 100% of the arsenic in all other foods was inorganic (Tao and Bolger 1999). Hand washing after play would reduce the potential exposure to children to arsenic after playing on play structures constructed with CCA-treated wood, since most of the arsenic on the children's hands was removed with water (Kwon et al. 2004).

Concentrations of several toxic metals, including the metalloid arsenic, were measured in the placentas of 200 women in two urban cities in Ukraine, Kyiv and Dniprodzerzhinsk. Arsenic was detected in only 5% of the samples with concentrations ranging from <0.156 to 0.378 μg/g. In a study in Bulgaria, placental arsenic concentrations of 7 and 23 μg/g were reported in a control and smelter area, respectively. A placental arsenic concentration of 34 μg/g was reported in a region of Argentina with high concentrations of arsenic in drinking water (Zadorozhnaja et al. 2000).

Parents can inadvertently carry hazardous materials home from work on their clothes, skin, hair, and tools, and in their vehicles (DHHS 1995). Falk et al. (1981b) reported a case of hepatic angiosarcoma in a child that could be associated with arsenic contamination of a parent's clothing, the water supply, and the environment. The father worked in a copper mine and smelter area where his clothing was contaminated with dust containing arsenic. His daughter, who exhibited a high degree of pica, ate soil from the yard, and licked soil off her father's shoes. In a study of arsenic levels in homes in Hawaii, Klemmer et al. (1975) found higher levels in homes of employees of firms that used arsenic for pesticides or wood preservation, compared to homes where residents' work did not involve arsenic. The concentration of arsenic in dust from the homes of workers exposed to arsenic ranged from 5.2 to 1,080 μg/g, compared to concentrations of 1.1–31 μg/g in dust from control homes.

While the harmful effects of many components of tobacco smoke are well known, those due to heavy metals in the smoke have not been sufficiently emphasized. The adverse health effects of these toxic metals on the fetus through maternal smoking are of special concern (Chiba and Masironi 1992). The concentration of arsenic in tobacco is relatively low, usually below detectable limits (<1 μg/g). Although the concentrations of inorganic and organic arsenic in the urine of adults do not appear to be influenced by smoking, a positive association was found between urinary arsenic levels in children and parental

smoking habits. As detailed in a WHO report, the mean arsenic level in the urine of children of nonsmoking parents was 4.2 μg/g creatinine, in children with one smoking parent, it was 5.5 μg/g, and in children with both parents smoking, it was 13 μg/g (Chiba and Masironi 1992). Tsuji et al. (2005) reported geometric mean concentrations of total arsenic of 15.1 and 15.7 μg/L in children <7 and <13 years old, respectively, from households in Middleport, New York, where historical pesticide manufacture was associated with arsenic in soil. Geometric mean concentrations of inorganic arsenic, MMA, and DMA were 0.81, 0.54, and 2.5 μg/L, respectively, in children <7 years old and 0.83, 0.55, and 3.0 μg/L, respectively, in children <13 years old (Tsuji et al. 2005).

The use of Chinese herbal medicines (CHM) appears to be common among Chinese women. Both CHM and Chinese proprietary medicines (CPM) are used for treatment of minor ailments in babies and children. Herbal medicines are available in capsule or tablet form in drug stores, supermarkets, and by mail. The CPM "Sin Lak Pill," "Lu Shen Wan," and other anti-asthma preparations have been found to contain inorganic arsenic levels ranging from 25 to 107,000 μg/g, and cases of acute arsenic poisoning have been found in children and adults using these CPM (Chan 1994). Babies and children are particularly at risk because they may be given higher doses of these preparations per kg of body weight than adults would normally consume. They may also lack the hepatic enzymes responsible for drug biotransformation and detoxification (Chan 1994). Concentrations of heavy metals, including arsenic, were evaluated in 54 samples of Asian remedies that were purchased in stores in Vietnam and Hong Kong that would be easily accessible to travelers, as well as in health food and Asian groceries in Florida, New York, and New Jersey. One remedy that was recommended to treat children's fever would expose a 15 kg child to approximately 5.0 mg of arsenic per day (Garvey et al. 2001). A folk remedy, purchased in California, for the treatment of chicken pox, flu-like symptoms, and nasal congestion, which had been given to two children in Wisconsin, was found to contain 36% arsenic acid. One-half teaspoon of this powder (about 500 mg of arsenic) was dissolved in hot water and taken 2–3 times per day (Werner et al. 2001).

Various metallic pigments and colors in the form of salts or lakes are used in toy production. Therefore, children may be exposed to toxic metals while playing with toys, especially when they lick, suck, or swallow a toy or a piece of a toy. Toys produced in European Union Markets must conform to restrictions concerning the bioavailability of toxic metals, including arsenic. The maximum limit for bioavailability of arsenic from the accessible parts of a toy is set to 0.1 μg/day. This corresponds to an arsenic migration limit of 25 μg/g for all toy material, including modeling clay and paints (Rastogi and Pritzl 1996). A study was carried out to determine whether crayons, water colors, and water-based paints

conform with the migration limits for toxic metals (Rastogi and Pritzl 1996). For the analysis, 94 samples representing 48 products were obtained from China, Taiwan, Japan, the United States, and European countries. Fifty-two samples showed migration of arsenic, ranging from 0.01 to 3.75 μg/g.

6.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

In addition to individuals who are occupationally exposed to arsenic (see Section 6.5), there are several groups within the general population that have potentially high exposures (higher than background levels) to arsenic. These populations include individuals living in proximity to sites where arsenic was produced, used (e.g., as a pesticide), or disposed, and individuals living near one of the 1,684 NPL hazardous waste sites where arsenic has been found at elevated levels in some environmental media (HazDat 2006). It also includes point sources such as smelters, coal-fired power plants, and municipal incinerators. People living in areas of volcanic activity may be exposed to higher levels of arsenic since high levels are more likely to be present in the environment. Other populations at risk of potentially high levels of exposure include those whose water supply contains high levels of arsenic and those consuming large amounts of seafood or seaweed. However, as pointed out previously (see Section 6.4.4), arsenic in fish and shellfish, is largely in the form of the less harmful organic arsenical, arsenobetaine; however, some commercially available seaweeds, especially brown algae varieties, may have high percentages of the total arsenic present as inorganic arsenic (>50%) (Almela et al. 2002; Laparra et al. 2003). While elevated urinary arsenic excretion levels have been associated with the consumption of fish and seafood, in a study of 32 sport fish consumers from Lakes Erie, Huron, and Michigan, only 6 (19%) had detectable urine arsenic concentrations, >4 μg/L, and 5 of these consumed fish from Lake Huron (Anderson et al. 1998). Exposure of high levels of arsenic in drinking water is more apt to be absorbed by the body and be harmful than exposure to arsenic in seafood. For example, a group of 36 people in Zimapán, Mexico who consumed water from an aquifer with 1.0 mg As/L had hair arsenic levels of 2.6–14.1 μg/g (10 μg/g average), compared with 2.4–13.9 μg/g (6.19 μg/g average) for a reference population that consumed bottled water with <0.014 mg/L arsenic (Armienta et al. 1997).

A study was conducted to determine if significant arsenic exposure was occurring at a Superfund site in Fort Valley, Georgia (Hewitt et al. 1995). Random urine, 24-hour urine, hair, and fingernail samples were collected at the end of the workweek from 40 employees at an active pesticide manufacturing facility where arsenical pesticides had been produced for over 50 years prior to the mid-1970s. Measurement of arsenic in the urine is considered to be the best method for monitoring recent exposure in industrial populations. Hair and fingernail analyses may provide an indication of exposures that occurred

up to several months prior to testing, but both can adsorb and strongly retain arsenic from external sources. Since arsenic is rapidly cleared from the blood (half-life of 3–4 hours), blood arsenic levels are not considered suitable for monitoring populations for chronic low-level arsenic exposure. Results of the Hewitt study are summarized in Table 6-6. Urinary arsenic levels for all workers were well within the commonly accepted normal range of <100 μg/L.

As noted above, workers in a number of industries may have high exposures to arsenic, especially if proper safety procedures are not followed. For members of the general population, above-average exposure to arsenic from drinking water is possible in areas of high natural arsenic levels in groundwater or elevated arsenic levels in drinking water due to industrial discharges, pesticide applications, or leaching from hazardous waste facilities. Individuals living in the vicinity of large smelters and other industrial emitters of arsenic may be exposed to above-average arsenic levels both in the air, and as a result of atmospheric deposition, in water and soil and subsequent uptake into crops.

People sawing or drilling arsenic-treated wood without protective masks or burning this wood may be exposed to elevated levels of arsenic in air.

Recreational and subsistence fishers who consume appreciably higher amounts of locally caught fish from contaminated bodies of water may be exposed to higher levels of arsenic associated with dietary intake. Arsenic contamination has triggered the issuance of several human health advisories (EPA 1998g). As of December 1997, arsenic was identified as the causative pollutant in a restricted consumption advisory for the general population for all fish in a 7-mile area including Devil's Swamp Lake and Bayou Baton Rouge in Louisiana. A public health advisory has been issued for consumption of fish and shellfish from the Duwamish River, Seattle, Washington due to arsenic and other chemicals (WSDOE 2005).

6.8 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of arsenic is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of arsenic.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.8.1 Identification of Data Needs

Physical and Chemical Properties. The chemical and physical properties of the arsenic species of chief toxicological and environmental concern are sufficiently well characterized to allow estimation of the environmental fates of these compounds. However, more information regarding the K_{ow} and K_{oc} values of the organic arsenicals would help predict the fate of these compounds in the environment.

Production, Import/Export, Use, Release, and Disposal. According to the Emergency Planning and Community Right-to-Know Act of 1986, 42 U.S.C. Section 11023, industries are required to submit substance release and off-site transfer information to the EPA. The TRI, which contains this information for 2004, became available in May of 2006. This database is updated yearly and should provide a list of industrial production facilities and emissions.

While arsenic has not been produced in the United States since 1985, the United States is the largest consumer of arsenic and substantial quantities of arsenic are imported, primarily as arsenic trioxide (USGS 2006a). The agricultural use of inorganic arsenic pesticides have been discontinued in the United States. However, some organic arsenicals still may be used in agriculture. Current production and use data for individual arsenical pesticides and other arsenic compounds would help to estimate human exposure to the various arsenic species. Because arsenical pesticides are so persistent, a more complete picture of past use of these products would enable us to predict what areas may contain high levels of arsenic in soil.

Comprehensive estimates on emissions of arsenic date to the early 1980s (Nriagu and Pacyna 1988). The industrial picture has changed considerably since then and emission controls are being mandated more and more. For example, emission factors for Canadian smelters calculated in 1993 were grossly lower than those estimated in 1983 (Skeaff and Dubreuil 1997). There is a need for accurate and up-to-date measurements of atmospheric arsenic releases from both natural and anthropogenic sources to better assess human exposure to arsenic and guide environmental protection measures.

Environmental Fate. The interconversion of the various arsenic species and transport among the environmental media is complex and not all aspects are well-studied. Additional quantitative data on the rates of oxidation, reduction, and biotransformation reactions of arsenic compounds, and how these depend on environmental conditions would be useful in evaluating and predicting the fate and transport of arsenic at hazardous waste sites and other areas.

Bioavailability from Environmental Media. Toxicokinetic and toxicity studies establish that bioaccessible (e.g., soluble, not strongly adsorbed to soil or embedded in minerals) arsenic is highly absorbed following inhalation and oral exposure (see Sections 3.4.1.2 and 3.4.1.1). Some work has been done on the effect of environmental matrix (soil, food) on accessibility and absorption of arsenic (Davis et al. 1992, 1996; Hamel et al. 1998; Roberts et al. 2002, 2007), but additional data would be valuable. Limited data suggests that dermal absorption of arsenic is very low (see Section 3.4.1.3) (Lowney et al. 2005), further data would be useful to establish whether arsenic uptake occurs from contact with contaminated soil or water, since humans may be exposed by these routes near hazardous waste sites.

Food Chain Bioaccumulation. Bioconcentration factors have been measured for several freshwater and marine species. While some species (mainly marine algae and shellfish) tend to bioconcentrate arsenic (EPA 1980a; Roper et al. 1996), it is not biomagnified through the food chain (Eisler 1994; EPA 1979, 1982b, 1983e, 2003b; Williams et al. 2006).

Carrots growing on land containing somewhat more than the permissible of arsenic in crop land did not contain levels of arsenic that were harmful (Helgesen and Larsen 1998). However, further research on the uptake of arsenic by a variety of plants in a wide range of arsenic polluted sites (e.g., mining area, orchards previously treated with lead arsenate) would be valuable in assessing human exposure near such sites through the consumption of vegetables from home gardens.

Exposure Levels in Environmental Media. Additional reliable monitoring data for the levels of arsenic in contaminated media at hazardous waste sites are needed or need to be made available, so that the information obtained on levels of arsenic in the environment can be used in combination with the known body burden of arsenic to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

Extensive monitoring data are available for total arsenic in all environmental media. Additional monitoring studies, specifically those that include identification of arsenic species, would allow more precise estimation of current exposure levels and possible human health risks.

Exposure Levels in Humans. Arsenic has been detected in human tissues, including blood, urine, hair, nails, and internal organs. Data are available for populations exposed in the workplace and for the general population (de Peyster and Silvers 1995; Jensen and Olsen 1995; Nygren et al. 1992), and some studies have been published on exposures near waste sites (Hwang et al. 1997a; Tsuji et al. 2005). Additional biomonitoring studies of residents near waste sites that contain arsenic would be helpful in evaluating the likely human health risks from these sites.

While some data are available on the speciation of arsenic in food, additional data on the particular species of arsenic, rather than just the total arsenic concentration, present in foods, especially seafood, are needed to better estimate the potential hazards to human health by the consumption of these foods (Ryan et al. 2001).

This information is necessary for assessing the need to conduct health studies on these populations.

Exposures of Children. Contaminated soils pose a particular hazard to children because of pica and hand-to-mouth activities. Some studies have been performed on exposure and body burden (Hwang et al. 1997a), but additional studies, including investigations of unique pathways for exposures of children and the amount of soil a child ingests, would provide valuable data. Small amounts of arsenic were found to be transferred to hands of children playing on play structures constructed from CCA-treated wood (Hemond and Solo-Gabriele 2004; Kwon et al. 2004; Shalat et al. 2006; Ursitti et al. 2004; Wang et al. 2005). Based on a review of existing studies, Hemond and Solo-Gabriele (2004) estimated that children with contact with CCA-treated wood may be subjected to doses in the range of tens of micrograms of arsenic per day and suggested that exposure by this route warrants further study. The PTDI assigned by the Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO) applies to adults. Studies are needed to assess whether children are different in their weight adjusted intake of arsenic. No childhood-specific means for reducing exposure were identified.

Child health data needs relating to susceptibility are discussed in Section 3.12.2, Identification of Data Needs: Children’s Susceptibility.

Exposure Registries. No exposure registries for arsenic were located. This substance is not currently one of the compounds for which a sub-registry has been established in the National Exposure Registry. The substance will be considered in the future when chemical selection is made for sub-registries to be established. The information that is amassed in the National Exposure Registry facilitates the epidemiological research needed to assess adverse health outcomes that may be related to exposure to this substance.

6.8.2 Ongoing Studies

The Federal Research in Progress (FEDRIP 2006) database provides additional information obtainable from a few ongoing studies that may fill in some of the data needs identified in Section 6.8.1. These studies are summarized in Table 6-7.

The U.S. Geological Survey, along with other federal and state agencies, industry, and academia, is conducting the National Geochemical Survey (NGS) in order to produce a body of geochemical data for the United States based primarily on stream sediments that have been analyzed using a consistent set of analytical methods. The goal of the NGS is to analyze at least one stream sediment sample in every 289 km^2 area by a single analytical method across the entire United States (USGS 2007b).

EPA is conducting a 4-year (2000–2003) national screening-level study of contaminants in freshwater fish, referred to as the National Fish Tissue Study (EPA 2004c). This study will allow the EPA to develop national estimates of the mean concentrations of 268 chemicals in tissues of fish from lakes and reservoirs of the coterminous United States. EPA analysis of the data from this study was scheduled to begin in January 2005, with the final report scheduled to be released in 2006. Interim raw data have been released each year, and are available from EPA. Fish samples have been analyzed for total inorganic arsenic (As(III) and As(V) combined), arsenic(III), arsenic(V), MMA(V), and DMA(V). Analysis for total arsenic was not performed as part of this study.

The American Water Works Association Research Foundation (AWWARF) supports research on arsenic in drinking water.

Table 6-7. Ongoing Studies on the Environmental Fate and Exposure of Humans to Arsenic

Investigator	Affiliation	Research description	Sponsor
Basta, NT	Ohio State University, School of Natural Resources, Columbus, Ohio	Heavy metal and trace element biogeochemistry in soils; chemical speciation, bioavailability, and toxicity	USDA
Blum, CB	Columbia University Health Sciences, New York, New York	Bioavailability lead and arsenic in soil to humans	NIEHS
Hamilton, JW	Dartmouth College, Hanover, New Hampshire	Toxic metals—biological and environmental implications	NIEHS
Hoppin, J	Not specified	Monitoring of arsenic and other compounds in the blood and urine of a cohort of pregnant women in Norway	NIEHS
Kpomblekou, AK; Ankumah, RO	Tuskegee University, Agriculture and Home Economics, Tuskegee, Alabama	Biochemical processes in soils treated with trace-element-enriched broiler litter; to determine total arsenic and other metal concentrations and the distribution of their chemical forms in soils under long-term broiler litter treatments	USDA
Loeppert, RH	Texas A&M University, Soil and Crop Sciences, College Station, Texas	Inorganic chemical processes influencing soil and water quality	USDA
Peryea, FJ	Wenatchee Tree Fruit Research & Extension Center Washington State University, Pullman, Washington	Quantification of biogeochemical processes in lead arsenate-contaminated orchard soils and development of soil and plant management practices to minimize the toxicity risks that these soils impose on agricultural crops and to human and environmental health	USDA
Miller, DM; DeLaune, P; Miller, WP	University of Arkansas, Crop, Soil and Environmental Sciences, Fayetteville, Arkansas	Arsenic levels in soils of northwest Arkansas	USDA
van Geen, A	Columbia University, Lamont-Doherty Earth Observatory, Palisades, New York	Studies on arsenic in groundwater in Bangladesh	NSF

Table 6-7. Ongoing Studies on the Environmental Fate and Exposure of Humans to Arsenic

Investigator	Affiliation	Research description	Sponsor
Walker, MJ et al.	University of Nevada, Natural Resources and Environmental Sciences, Reno, Nevada	Arsenic in Churchill County, Nevada domestic water supplies	USDA
Zheng, Y	Columbia University Health Sciences, New York, New York	Arsenic mobilization in Bangladesh groundwater	NIEHS

NIEHS = National Institute of Environmental Health Sciences; NSF = National Science Foundation; USDA = U.S. Department of Agriculture

Source: FEDRIP 2006

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7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring arsenic, its metabolites, and other biomarkers of exposure and effect to arsenic. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision.

7.1 BIOLOGICAL MATERIALS

Atomic absorption spectrophotometry (AAS) is the most common analytical procedure for measuring arsenic in biological materials (Curatola et al. 1978; Foà et al. 1984; Johnson and Farmer 1989; Mushak et al. 1977; Norin and Vahter 1981; Sotera et al. 1988). In AAS analysis, the sample is heated in a flame or in a graphite furnace until the element atomizes. The ground-state atomic vapor absorbs monochromatic radiation from a source and a photoelectric detector measures the intensity of transmitted radiation (APHA 1989b). Inductively-coupled plasma atomic emission spectrometry (ICP-AES) and ICP-mass spectrometry (ICP-MS) are increasingly common techniques for the analysis of arsenic; both methods can generally provide lower detection limits than absorbance detection methods.

Samples may be prepared for AAS in a variety of ways. Most often, the gaseous hydride procedure is employed (Curatola et al. 1978; Foà et al. 1984; Johnson and Farmer 1989; Norin and Vahter 1981). In this procedure, arsenic in the sample is reduced to arsine (AsH_3), a gas that is then trapped and introduced into the flame. This approach measures total inorganic arsenic, but may not detect all organic forms unless preceded by a digestion step. Digestion or wet-ashing with nitric, sulfuric, and/or perchloric acids degrades the organic arsenic species to inorganic arsenic so that recovery of total arsenic from biological materials can be achieved (Maher 1989; Mushak et al. 1977; Versieck et al. 1983). In microwave assisted digestion, harsh oxidation conditions are used in conjunction with microwave heating (Benramdane et al. 1999b). For accurate results, it is important to check the completeness of the oxidation; however, this is seldom done (WHO 1981).

The arsenic concentration in biological fluids and tissues may also be determined by neutron activation analysis (NAA) (Landsberger and Simsons 1987; Versieck et al. 1983). In this approach, the sample is irradiated with a source of neutrons that converts a portion of the arsenic atoms to radioactive isotopes, which can be quantified after separation from radioisotopes of other chemicals. Neutron activation has limited use because of the limited number of nuclear reactors in the United States providing this service and the need to dispose of radioactive waste. X-ray fluorescence is also capable of measuring arsenic in biological materials (Bloch and Shapiro 1986; Clyne et al. 1989; Nielson and Sanders 1983) and environmental samples (see Section 7.2). This method has the advantage that no sample digestion or separation steps are required. Hydride generation combined with atomic fluorescence spectroscopy (HG-AFS) is a relatively new technique that provides freedom from interference offered by hydride generation with sensitivity better than to 20 parts per trillion and linearity up to 10 ppm (PSA 2000).

Speciation of arsenic (i.e., analysis of organic arsenic compounds or different inorganic species, rather than total arsenic) is usually accomplished by employing separation procedures prior to introduction of the sample material into a detection system. Various types of chromatography or chelation-extraction techniques are most commonly used in combination with AAS, ICP-AES, or ICP-MS detection methods (Dix et al. 1987; Foà et al. 1984; Johnson and Farmer 1989; Mushak et al. 1977; Norin et al. 1987; Thomas and Sniatecki 1995). In one method, high performance liquid chromatography (HPLC) is combined with HG-AFS to quantify As(III), dimethylarsinic acid (DMA), momomethyl arsonic acid (MMA), and As(V) (PSA 2000). Another approach involves selective reduction of arsenate and arsenite (permitting quantification of individual inorganic arsenic species), and selective distillation of methyl arsines to quantify MMA and DMA (Andreae 1977; Braman et al. 1977; Crecelius 1978). Most methods for measuring arsenic in biological samples are unable to measure arsenobetaine with any accuracy because it does not form a hydride and it gives a different response from inorganic arsenic in electrothermal AAS. Ebdon et al. (1999) successfully employed HPLC coupled with ICP-MS to determine arsenic speciation in blood plasma, which was entirely arsenobetaine. Øygard et al. (1999) developed a simple method to determine inorganic arsenic in biological samples. Their method, which involves initially distilling inorganic arsenic from the sample as $AsCl_3$ using HCl, avoids separating and quantifying all of the different arsenic species, which is both costly and time-consuming.

Table 7-1 summarizes a variety of methods for measuring total arsenic and individual arsenic species in biological materials. None of these methods have been standardized by EPA or other federal agencies.

Table 7-1. Analytical Methods for Determining Arsenic in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Methods for total arsenic:					
Blood	Digestion with nitric acid and hydrogen peroxide; dry ash with magnesium oxide/magnesium nitrate; reduction with sodium borohydride	HGAAS	0.5 μg/L	95–102	Foà et al. 1984
Blood, hair	Wet ash with nitric/perchloric acids; reduction with sodium borohydride	HGAAS	0.1 μg/L[a]	95–105	Valentine et al. 1979
Serum	Irradiation; digestion with nitric/ perchloric/sulfuric acids; extraction with toluene	NAA	0.088 ng/mL[a]	94–98	Versieck et al. 1983
Urine	Irradiate epithermally	NAA	40–100 ng/g	93–109	Landsberger and Simsons 1987
Urine	Digestion with nitric and perchloric acid; reduction with tin chloride; generation arsine by addition of zinc; reaction with SDDC	Colorimetric photometry	0.5 μg/sample	90–110	Pinto et al. 1976
Urine	Pretreatment with L-cysteine; reduction with potassium iodide/ ascorbic acid	Flow injection HGAAS	0.1 μg/L	95–100	Guo et al. 1997
Urine	Drying sample; irradiation with x-rays	XRF	0.2 μg/L[a]	92–108	Clyne et al. 1989
Hair	Wet ashing with nitric/sulfuric acids and hydrogen peroxide; reduction to arsine with sodium borohydride	HGAAS	0.06 μg/g	93	Curatola et al. 1978
Soft tissue	Digestion with nitric/sulfuric acids; complexation with DDDC in potassium iodide; extraction with chloroform	GFAAS	0.2 ppm	79.8	Mushak et al. 1977
Nails	Wet ashing with nitric/sulfuric acids and hydrogen peroxide; reduction to arsine with sodium borohydride	HGAAS	1.5 μg/g	No data	Agahian et al. 1990
Methods for arsenic speciation:					
Urine	Separation of As^{+3}, As^{+5}, MMA, and DMA on anion/cation exchange resin column; reduction to respective arsines with sodium borohydride	IEC/HGAAS	0.5 μg/L	93–106	Johnson and Farmer 1989
Urine	Reduction of As^{+3}, As^{+5}, MMA, and DMA to arsines with sodium borohydride	HGAAS	0.08 μg/L	97–104	Norin and Vahter 1981

Table 7-1. Analytical Methods for Determining Arsenic in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine	Reduction of As^{+3}, As^{+5}, MMA, and DMA to arsines; collection in cold trap; selective distillation by slow warming	Atomic emission (direct-current plasma)	≤1 ng for all four species	No data	Braman et al. 1977
Urine	Extraction with chloroform/ methanol; column separation with chloroform/methanol; elution on cation exchange column with ammonium hydroxide	HGAAS/TLC/ HRMS	0.34 mg/ sample[a]	No data	Tam et al. 1982
Blood/ tissue	Acidification with hydrochloric acid; complexation with TGM; extraction into cyclohexane; separation on capillary column	GLC/ECD	0.1 mg/mL	No data	Dix et al. 1987
Blood plasma	Separation by HPLC	HPLC/ICP-MS	2.5 ng As/mL	~100	Ebdon et al. 1999
Urine	Separation by anion exchange chromatography; detection by direct coupling of column to ICP-MS	IEC/ICP-MS	<0.45 μg/L for all species	No data	Inoue et al. 1994
Marine biota	Extraction with methanol-water; removal of fats by liquid-liquid extraction or solid-phase cartridge	HPLC/ICP-MS	6–25 ng/mL	94.6 (fish muscle CRM)	Sniatecki 1994
Marine biota	Separation by anion exchange coupled with HPLC; on-line microwave oxidation	HPLC/ HGAAS	0.3–0.9 ng	95–110 (recovery of spike in fish tissue)	López-Gonzálvez et al. 1994
Biological samples—Inorganic arsenic	Distill inorganic arsenic as $AsCl_3$ using HCl after prereduction of As(V) with KI/HCl	Flow-injection HGAAS	0.045 mg/kg (dry matter)	No data	Øygard et al. 1999

[a]Lowest reported concentration

CRM = certified reference material; DDDC = diethylammonium diethyldithiocarbamate; DMA = dimethylarsinate; ECD = electron capture detector; GFAAS = graphite furnace atomic absorption spectrometry; GLC = gas-liquid chromatography; HGAAS = hydride generation atomic absorption spectrometry; HRMS = high resolution mass spectrometry; ICP-MS = inductively-coupled plasma mass spectrometry; IEC = ion exchange chromatography; HPLC = high-performance liquid chromatography; MMA = monomethylarsonate; NAA = neutron activation analysis; SDDC = silver diethyldithiocarbamate; TGM = thioglycolic acid methylester; TLC = thin layer chromatography; XRF = x-ray fluorescence

Detection limits in blood and urine are about 0.1–1 ppb for most techniques; limits for hair and tissues are usually somewhat higher.

7.2 ENVIRONMENTAL SAMPLES

Arsenic in environmental samples is also measured most often by AAS techniques, with samples prepared by digestion with nitric, sulfuric, and/or perchloric acids (Dabeka and Lacroix 1987; EPA 1983b, 1994a, 1994b; Hershey et al. 1988). Other methods employed include a spectrophotometric technique in which a soluble red complex of arsine and silver diethyldithiocarbamate (SDDC) is formed (APHA 1977; EPA 1983c, 1983d), ICP-AES (EPA 2000c; NIOSH 2003), graphite furnace AAS (EPA 1983b, 1994b; NIOSH 1994b), ICP-MS (EPA 1991, 1994a, 1998j), and x-ray fluorescence (Khan et al. 1989; Nielson and Sanders 1983).

HPLC is currently the most common technique for separation of the species of arsenic found in seafood (Benramdane et al. 1999b; Guerin et al. 1999; Kumaresan and Riyazuddin 2001). An advantage of HPLC over other separation methods (e.g., gas chromatography [GC]) is that the arsenic species do not need to be derivatized prior to separation, avoiding concerns over complete conversion to the derivative for detection.

Since arsenic in air is usually associated with particulate matter, standard methods involve collection of air samples on glass fiber or membrane filters, acid extraction of the filters, arsine generation, and analysis by SDDC spectrophotometry or AAS (APHA 1977; NIOSH 1984).

Methods standardized by the EPA for measuring total arsenic in water and waste water, solid wastes, soil, and sediments include: ICP-MS (EPA 1998j, 1994a, 1991), ICP-AES (EPA 1996d), graphite furnace AAS (EPA 1994b), quartz furnace hydride generation AAS (EPA 1996h), and an electrochemical method using anodic stripping voltammetry (ASV) (EPA 1996e). A modification using cryogenic GC to EPA Method 1632 (HG/AAS) allows the technique to be adopted for the species As(III), As(V), MMA, and DMA to the 0.003 ppb level (EPA 1998l). Similar methods are recommended by APHA for water using AAS/hydride generation (APHA 1989c), AAS/graphite furnace technique (APHA 1989b), ICP (APHA 1989d), or SDDC spectrophotometry (APHA 1989a). The AAS/hydride generation method is generally resistant to matrix and chemical interferences (APHA 1989a). Techniques to compensate for these interferences have been described by EPA (1982b).

Analysis for arsenic in foods is also most frequently accomplished by AAS techniques (Arenas et al. 1988; Dabeka and Lacroix 1987; Hershey et al. 1988; Tam and Lacroix 1982). Hydride generation is the sample preparation method most often employed (Arenas et al. 1988; Hershey et al. 1988), but interferences must be evaluated and minimized.

Speciation of inorganic arsenic in environmental samples is usually accomplished by chromatographic separation, chelation-extraction or elution of As(III), and then reduction of As(V) with subsequent similar treatment (Butler 1988; López-Gonzálvez et al. 1994; Mok et al. 1988; Rabano et al. 1989).

Methods are also available for quantifying organic arsenicals in environmental media, including arsenobetaine in fish (Beauchemin et al. 1988; Cannon et al. 1983) and other organic forms of arsenic in water, soil, and foods using hyphenated methods of separation and detection (HPLC/ICP-MS, HPLC/HGAAS, IC/ICP-MS) (Andreae 1977; Braman et al. 1977; Comber and Howard 1989; Crecelius 1978; Heitkemper et al. 1994; López-Gonzálvez et al. 1994; Odanaka et al. 1983; Teräsahde et al. 1996).

Methods have been developed for extraction of arsenic species from solid seafood samples that included treatment of the sample with mixtures of organic solvents (alcohols or chloroform) and water to extract the arsenic compounds that are soluble in water or polar organic solvents. These extracts can be subsequently analyzed by HPLC. Enzymatic digestion using trypsin has also been used to extract arsenic compounds from seafood samples (Benramdane et al. 1999b). These extraction techniques are used in place of digestion when speciated data are needed.

A summary of selected methods for analysis of total arsenic and individual inorganic and organic arsenic species in environmental samples is presented in Table 7-2.

7.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of arsenic is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of arsenic.

Table 7-2. Analytical Methods for Determining Arsenic in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Methods for total arsenic:					
Air (particulates)	Collection on cellulose ester membrane filter; digestion with nitric acid, sulfuric acid, and perchloric acid	NIOSH Method 7900; HGAAS	0.02 μg/sample	No data	NIOSH 1994a
Air (particulate arsenic and arsenic trioxide vapor)	Collection on Na_2CO_3-impregnated cellulose ester membrane filter and H_2O_2	NIOSH Method 7901; GFAAS	0.06 μg/sample	No data	NIOSH 1994b
Air	Collection on cellulose ester membrane filter; digestion with nitric acid, sulfuric acid, and perchloric acid	NIOSH Method 7300; ICP-AES	0.140 μg/filter	No data	NIOSH 2003
Water/waste water/solid wastes	Acid digestion	EPA Method 6010C; ICP-AES	35 μg/L	86	EPA 2000c
Water/waste water/solid wastes	Digestion with nitric and hydrochloric acids	EPA Method 200.7; ICP-AES	8 μg/L	106	EPA 1994c
Water/soil/ solid waste	Digestion with nitric acid and hydrogen peroxide	EPA Methods 206.2 and 7060A; GFAAS with $Ni(NO_3)_2$ modifier	1 μg/L	85–106	EPA 1983b, 1994b
Water/waste water/solid waste	Digestion with nitric acid	EPA Methods 200.8, 6020 and 6020A ICP-MS	0.4 μg/L	97–114	EPA 1991, 1994a, 1998j
Water/soil/ solid waste	Digestion with nitric/sulfuric acid; reduction to As^{+3} with tin chloride; reduction to arsine with zinc in acid solution	EPA Method 206.3	2 μg/L	85–94	EPA 1983c
Water	Reduction to arsine in acid solution; reaction with SDDC	EPA Method 206.4; SDDC colorimetric spectrophoto-metry at 510 nm	10 μg/L	100	EPA 1983d
Water	Digestion with 6M HCl; reduction to arsine with sodium borohydride; cold trap and desorption into quartz furnace	EPA Method 1632; HGAAS	2 ng/L	No data	EPA 1998l

Table 7-2. Analytical Methods for Determining Arsenic in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Food	Digestion with nitric acid; dry ashing with magnesium oxide; reduction with ascorbic acid; precipitation with APDC in presence of nickel carrier	GFAAS	10 ng	86–107	Dabeka and Lacroix 1987
Food	Digestion with nitric/sulfuric/ perchloric acids; reduction to trivalent arsenic with potassium iodide; reduction to arsine with sodium borohydride	HGAAS	0.1 μg/g	98–110	Hershey et al. 1988
Soil, rock, coal	Preparation of pellet	XRF (backscatter)	4 mg/kg	SRM recoveries: 110±4 in soil; 100±1in rock; 97±18 in coal	Nielson and Sanders 1983
Methods for species of arsenic:					
Air (particulate organo-arsenals)	Collection on PTFE filter	NIOSH Method 5022; ion chromatography/HGAAS	0.2 μg As/sample	No data	NIOSH 1994c
Air (arsine)	Collection on coconut shell charcoal; digestion with nitric acid	NIOSH Method 6001; GFAAS	0.004 μg/sample	No data	NIOSH 1994d
Air particulates (As^{+3} and As^{+5} only)	Collection on PFTE filter in high volume dichotomous virtual impactor; desorption with ethanolic hydrochloric acid; selective reduction of As^{+3} to arsine with zinc in acid and reduction of As^{+5} to arsine with sodium tetrahydrodiborate	HGAAS	1 ng/m^3	95±7 (As^{+3}); 100±8 (As^{+5}) on spiked materials	Rabano et al. 1989
Water	Selective elution of As^{+3} with orthophosphoric acid; elution and conversion of As^{+5} to As^{+3} with sulfur dioxide	IEC/amperometric detector (detects As^{+3} only)	0.9 μg/L	95% of converted As^{+5} recovered	Butler 1988
Water/soil	Selective complexation of As^{+5} with ammonium molybdate; extraction with isoamyl alcohol to separate from As^{+3}	Colorimetric spectrometry at 712 nm	No data	No data	Brown and Button 1979

Table 7-2. Analytical Methods for Determining Arsenic in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Selective extraction of As^{+3} with APDC into chloroform; back extraction with nitric acid; reduction of As^{+5} to As^{+3} with thiosulfate and extract	NAA	0.01 ppb	No data	Braman et al. 1977
Food (arseno-betaine in fish)	Extraction of arsenobetaine with methanol/chloroform; digestion with nitric acid/ magnesium nitrate for remainder of As species	HPLC/ICP-MS	0.3 ng as arsenobetaine	101±4 recovery of arseno-betaine	Beauchemin et al. 1988
Water/waste water/soil (inorganic species)	Acidification or digestion with hydrochloric acid	EPA Method 7063; ASV	0.1 μg/L	96–102	EPA 1996e
Water (As(III), As(V), MMA, and DMA)	Cryogenic GC, Digestion with 6M HCl; reduction to arsine with sodium boro-hydride; cold trap and desorption into quartz furnace	EPA Method 1632 appendix; HGAAS	3 ng/L	No data	EPA 1998l
Water	Reduction to arsines; cold trap and selectively warm to separate arsine species	AAS	2 ng/L	91–109	Andreae 1977
Water	Reduction of MMA, DMA and inorganic As (control pH to select As^{+3} or As^{+5}) to arsines with sodium tetra-hydroborate; cold trap and selectively warm to separate arsine species	HGAAS	0.019–0.061 ng	No data	Comber and Howard 1989
Water/soil	Extraction with sodium bicarbonate; reduction of inorganic arsenic, MMA and DMA to hydrides with sodium borohydride; cold trap arsines in n-heptane	HG-HCT/GC-MID	0.2–0.4 μg/L	97–102	Odanaka et al. 1983

AAS = atomic absorption spectrophotometry; APDC = ammonium pyrrolidine dithiocarbamate; ASV = anodic stripping voltammetry; DMA = dimethylarsinate; EPA = Environmental Protection Agency; GC-MID = gas chromatography-multiple ion detection; GFAAS = graphite furnace atomic absorption spectrometry; HGAAS=hydride generation-atomic absorption spectroscopy; HG-HCT = hydride generation-heptane cold trap; HPLC = high performance liquid chromatography; ICP-AES = inductively coupled plasma-atomic emission spectrometry; ICP-MS = inductively coupled plasma-mass spectrometry; IEC = ion exchange chromatography; MMA = monomethylarsonate; NAA = neutron activation analysis; NIOSH = National Institute of Occupational Safety and Health; PTFE = polytetrafluoroethylene; SDDC = silver diethyldithiocarbamate; SRM = standard reference material; XRF = x-ray fluorescence

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. The most common biomarker for arsenic exposure is analysis of total arsenic in urine (Hughes 2006). Existing methods are sufficiently sensitive to measure background levels of arsenic in various tissues and biological fluids for average persons, and to detect increases as a result of above-average exposure (Agahian et al. 1990; Clyne et al. 1989; Curatola et al. 1978; Foà et al. 1984; Gebel et al. 1998b; Landsberger and Simsons 1987; Mushak et al. 1977; Pinto et al. 1976; Valentine et al. 1979; Versieck et al. 1983). The precision and accuracy of these methods are documented. Methods are also available that can distinguish nontoxic forms of arsenic (arsenobetaine) from inorganic and organic derivatives that are of health concern (Braman et al. 1977; Dix et al. 1987; Johnson and Farmer 1989; Norin and Vahter 1981; Tam et al. 1982). Further efforts to improve accuracy, reduce interferences, and detect multiple species using a single analysis would be valuable. Arsenic is believed to act by inhibition of numerous cellular and molecular processes. However, these effects are not specific to arsenic, and most can only be measured in tissue extracts.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Arsenic is ubiquitous in the environment. It is found in air, water, soil, sediments, and food in several inorganic and organic forms. Analytical methods exist for the analysis of arsenic species in all of these environmental media, and these methods have the sensitivity to measure background levels and to detect elevated concentrations due to emissions from sources such as smelters, chemical plants, or hazardous waste sites (APHA 1977, 1989c; EPA 1982b, 1983b, 1983c, 1983d, 1991, 1994b, 1994c, 1996f, 1996h, 1998j, 2000c; NIOSH 1994a, 1994b, 2003). However, further research to reduce chemical and matrix interferences may improve the speed and accuracy of the analyses.

Le et al. (2004) pointed out that there is a need for the development of certified reference materials (CRMs) for speciation analysis. A shortcoming of many CRMs is that they are only certified for the total concentration of arsenic, and only limited information is available on the identity and concentrations of specific arsenic species in some CRMs.

Continued improvement of the methods for determination of the particular species of arsenic, rather than just the total arsenic concentration, present in foods, especially seafood, is needed since different arsenic species poses different hazards to individuals consuming these foods.

7.3.2 Ongoing Studies

The information in Table 7-3 was found as a result of a search of the Federal Research in Progress database (FEDRIP 2006).

Table 7-3. Ongoing Studies on Analytical Methods for Arsenic in Environmental and Biological Samples

Investigator	Affiliation	Research description	Sponsor
Styblo, M	University of North Carolina Chapel Hill, Chapel Hill, North Carolina	Optimized hydride generation system for arsenic analysis	Fogarty International Center
Dietze, WT	Tracedetect, Inc., Seattle, Washington	A continuous monitor for arsenic in drinking water	NIEHS
Dasgupta, PK	Texas Tech University, Department of Chemistry, Lubbock, Texas	A green fieldable analyzer for arsenic	NSF

NIEHS = National Institute of Environmental Health Sciences; NSF = National Science Foundation

Source: FEDRIP 2006

8. REGULATIONS AND ADVISORIES

The international and national regulations and guidelines pertaining to arsenic and its metabolites in air, water, and other media are summarized in Table 8-1.

ATSDR has not derived inhalation MRLs or an intermediate-duration oral MRL for inorganic arsenic, or any MRLs for organic arsenic, due to lack of suitable data.

ATSDR has derived an acute-duration oral MRL for inorganic arsenic of 0.005 mg As/kg/day based on a LOAEL of 0.05 mg As/kg/day for gastrointestinal effects and facial edema in Japanese people who ingested arsenic-contaminated soy sauce for 2–3 weeks (Mizuta et al. 1956). An uncertainty factor of 10 (10 for use of a LOAEL and 1 for human variability) was applied.

ATSDR has derived a chronic-duration oral MRL of 0.0003 mg/kg/day for inorganic arsenic based on a NOAEL of 0.0008 mg As/kg/day for dermal effects in a Taiwanese farming population exposed to arsenic in well water (Tseng 1977; Tseng et al. 1968). An uncertainty factor of 3 (for human variability) was applied.

EPA (IRIS 2007) has derived a chronic oral reference dose (RfD) of 0.0003 mg As/kg/day for inorganic arsenic, based on a NOAEL of 0.0008 mg As/kg/day for dermal effects and possible vascular complications in a Taiwanese farming population exposed to arsenic in well water (Tseng 1977; Tseng et al. 1968). An uncertainty factor of 3 (to account for the lack of reproductive data and uncertainty in whether the NOAEL accounts for all sensitive individuals) was applied. No reference concentration (RfC) for chronic inhalation exposures to arsenic was reported. EPA is currently revising the assessment for inorganic arsenic.

The Department of Health and Human Services (DHHS) has determined that inorganic arsenic is known to be a human carcinogen (NTP 2005). The EPA has determined that inorganic arsenic is a human carcinogen and has assigned it the cancer classification, Group A (IRIS 2007). EPA's quantitative estimates of carcinogenic risk from oral exposures include a cancer slope factor of 1.5 mg/kg/day and a drinking water unit risk of $5x10^{-5}$ μg/L. The inhalation unit risk for cancer is 0.0043 $\mu g/m^3$ (IRIS 2007).

Table 8-1. Regulations and Guidelines Applicable to Arsenic and Arsenic Compounds

Agency	Description	Information	Reference
INTERNATIONAL			
Guidelines:			
IARC	Carcinogenicity classification for arsenic and arsenic compounds	Group 1[a]	IARC 2004
WHO	Air quality guidelines	1.5×10^{-3} unit risk[b]	WHO 2000
	Drinking water quality guidelines for arsenic	0.01 mg/L[c]	WHO 2004
NATIONAL			
Regulations and Guidelines:			
a. Air			
ACGIH	TLV (TWA) for arsenic and inorganic compounds	0.01 mg/m^3	ACGIH 2004
EPA	Hazardous air pollutant (arsenic and inorganic compounds, including arsine)	Yes	EPA 2004b 42 USC 7412
NIOSH	REL (15-minute ceiling limit) for arsenic and inorganic compounds[d]	0.002 mg/m^3	NIOSH 2005a
	IDLH for arsenic and inorganic compounds[d]	5 mg/m^3	
OSHA	PEL (8-hour TWA) for general industry for arsenic organic compounds	0.5 mg/m^3	OSHA 2005d 29 CFR 1910.1000
	PEL (8-hour TWA) for general industry for arsenic inorganic compounds	10 µg/m^3	OSHA 2005c 29 CFR 1910.1018
	PEL (8-hour TWA) for construction industry for arsenic organic compounds	0.5 mg/m^3	OSHA 2005b 29 CFR 1926.55
	PEL (8-hour TWA) for shipyard industry for arsenic organic compounds	0.5 mg/m^3	OSHA 2005a 29 CFR 1915.1000
b. Water			
EPA	Designated as hazardous substances in accordance with Section 311(b)(2)(A) of the Clean Water Act	Yes	EPA 2005d 40 CFR 116.4
	Arsenic pentoxide, arsenic trioxide, calcium arsenate, and sodium arsenite		
	Drinking water standards and health advisories for arsenic		EPA 2004a
	DWEL	0.01 mg/L	
	National primary drinking water standards for arsenic		EPA 2002a
	MCLG	Zero	
	MCL	0.01 mg/L[e]	
	Reportable quantities of hazardous substances designated pursuant to Section 311 of the Clean Water Act		EPA 2005e 40 CFR 117.3
	Arsenic pentoxide, arsenic trioxide, calcium arsenate, sodium arsenite	1 pound	

Table 8-1. Regulations and Guidelines Applicable to Arsenic and Arsenic Compounds

Agency	Description	Information	Reference
NATIONAL *(cont.)*			
EPA	Water quality criteria for human health consumption of arsenic:		EPA 2002b
	Water + Organism	0.018 μg/L[f]	
	Organism only	0.14 μg/L[f]	
c. Food			
EPA	Tolerances for residues		
	Dimethylarsinic acid		EPA 2005i 40 CFR 180.311
	Cotton (undelinted seed)	2.8 ppm	
	Methanearsonic acid		EPA 2005j 40 CFR 180.289
	Cotton (undelinted seed)	0.7 ppm	
	Cotton, hulls	0.9 ppm	
	Fruit, citrus	0.35 ppm	
FDA	Bottled drinking water	0.01 mg/L	FDA 2005 21 CFR 165.110
USDA	Nonsynthetic substances prohibited for use in organic crop production	Arsenic	USDA 2004 7 CFR 205.602
d. Other			
ACGIH	Carcinogenicity classification for arsenic and arsenic compounds	A1[g]	ACGIH 2004
	Biological exposure indices for inorganic arsenic plus methylated metabolites in urine at the end of the workweek	35 μg As/L	
EPA	Carcinogenicity classification	Group A[i]	IRIS 2007
	Oral slope factor	1.5 per mg/kg/day	
	Inhalation unit risk	4.3×10^{-3} per μg/m^3	
	RfC	No data	
	RfD	3×10^{-4} mg/kg/day	
	Superfund, emergency planning, and community right-to-know		
	Designated CERCLA hazardous substance		EPA 2005f 40 CFR 302.4
	Reportable quantity		
	Arsenic	Not applicable[j]	
	Arsenic acid, arsenic pentoxide, arsenic trioxide, calcium arsenate, dimethylarsinic acid, and sodium arsenite	1 pound	
	Effective date of toxic chemical release reporting for arsenic	01/01/87	EPA 2005h 40 CFR 372.65

Table 8-1. Regulations and Guidelines Applicable to Arsenic and Arsenic Compounds

Agency	Description	Information	Reference
NATIONAL *(cont.)*			
EPA	Superfund, emergency planning, and community right-to-know		
	Extremely hazardous substances		EPA 2005g 40 CFR 355, Appendix A
	Reportable quantity		
	Arsenic pentoxide, calcium arsenate, and sodium arsenite	1 pound	
	Threshold planning quantities		
	Arsenic pentoxide	100/10,000 pounds	
	Calcium arsenate and sodium arsenite	500/10,000 pounds	
NTP	Carcinogenicity classification	Known human carcinogen	NTP 2005

[a]Group 1: carcinogenic to humans
[b]Cancer risk estimates for lifetime exposure to a concentration of 1 μg/m3.
[c]Provisional guideline value: as there is evidence of a hazard, but the available information on health effects is limited.
[d]NIOSH potential occupational carcinogen
[e]MCL will become effective on 01/23/06.
[f]This criterion is based on carcinogenicity of 10^{-6} risk.
[g]A1: confirmed human carcinogen
[h]A3: confirmed animal carcinogen with unknown relevance to humans
[i]Group A: known human carcinogen
[j]Indicates that no reportable quantity is being assigned to the generic or broad class.

ACGIH = American Conference of Governmental Industrial Hygienists; CERCLA = Comprehensive Environmetnal Response, Compensation, and Liability Act; CFR = Code of Federal Regulations; DWEL = drinking water equivalent level; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; IARC = International Agency for Research on Cancer; IDLH = immediately dangerous to life or health; IRIS = Integrated Risk Information System; MCL = maximum contaminant level; MCLG = maximum contaminant level goal; NAS/NRC = National Academy of Sciences/National Research Council; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; PEL = permissible exposure limit; REL = recommended exposure limit; RfC = inhalation reference concentration; RfD = oral reference dose; TLV = threshold limit values; TWA = time-weighted average; USC = United States Code; USDA = United States Department of Agriculture; WHO = World Health Organization

EPA is currently revising the assessment for inorganic arsenic. The International Agency for Research on Cancer (IARC) cites sufficient evidence of a relationship between exposure to arsenic and human cancer. IARC classification of arsenic is Group 1 (IARC 2004). The American Conference of Governmental Industrial Hygienists (ACGIH) classifies arsenic (elemental and inorganic compound) as a confirmed human carcinogen, cancer category A1 (ACGIH 2004).

ATSDR has derived an intermediate-duration oral MRL of 0.1 mg MMA/kg/day for MMA based on a $BMDL_{10}$ of 12.38 mg MMA/kg/day for diarrhea observed in rats exposed to MMA in the diet for 13 weeks (Arnold et al. 2003) and an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability).

ATSDR has derived a chronic-duration oral MRL of 0.01 mg MMA/kg/day for MMA based on a $BMDL_{10}$ of 1.09 mg MMA/kg/day for increased incidence of progressive nephropathy in male mice exposed to MMA in the diet for 2 years (Arnold et al. 2003) and an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability).

ATSDR has derived a chronic-duration oral MRL of 0.02 mg DMA/kg/day for DMA based on a $BMDL_{10}$ of 1.80 mg DMA/kg/day for increased vacuolization of the urothelium in the urinary bladder of female mice exposed to DMA in the diet for 2 years (Arnold et al. 2006) and an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability).

EPA has not derived RfD values for organic arsenicals (IRIS 2007).

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10. GLOSSARY

Absorption—The taking up of liquids by solids, or of gases by solids or liquids.

Acute Exposure—Exposure to a chemical for a duration of 14 days or less, as specified in the Toxicological Profiles.

Adsorption—The adhesion in an extremely thin layer of molecules (as of gases, solutes, or liquids) to the surfaces of solid bodies or liquids with which they are in contact.

Adsorption Coefficient (K_{oc})—The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

Adsorption Ratio (Kd)—The amount of a chemical adsorbed by sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

Benchmark Dose (BMD)—Usually defined as the lower confidence limit on the dose that produces a specified magnitude of changes in a specified adverse response. For example, a BMD_{10} would be the dose at the 95% lower confidence limit on a 10% response, and the benchmark response (BMR) would be 10%. The BMD is determined by modeling the dose response curve in the region of the dose response relationship where biologically observable data are feasible.

Benchmark Dose Model—A statistical dose-response model applied to either experimental toxicological or epidemiological data to calculate a BMD.

Bioconcentration Factor (BCF)—The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

Biomarkers—Broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility.

Cancer Effect Level (CEL)—The lowest dose of chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or tumors) between the exposed population and its appropriate control.

Carcinogen—A chemical capable of inducing cancer.

Case-Control Study—A type of epidemiological study that examines the relationship between a particular outcome (disease or condition) and a variety of potential causative agents (such as toxic chemicals). In a case-controlled study, a group of people with a specified and well-defined outcome is identified and compared to a similar group of people without outcome.

Case Report—Describes a single individual with a particular disease or exposure. These may suggest some potential topics for scientific research, but are not actual research studies.

Case Series—Describes the experience of a small number of individuals with the same disease or exposure. These may suggest potential topics for scientific research, but are not actual research studies.

Ceiling Value—A concentration of a substance that should not be exceeded, even instantaneously.

Chronic Exposure—Exposure to a chemical for 365 days or more, as specified in the Toxicological Profiles.

Cohort Study—A type of epidemiological study of a specific group or groups of people who have had a common insult (e.g., exposure to an agent suspected of causing disease or a common disease) and are followed forward from exposure to outcome. At least one exposed group is compared to one unexposed group.

Cross-sectional Study—A type of epidemiological study of a group or groups of people that examines the relationship between exposure and outcome to a chemical or to chemicals at one point in time.

Data Needs—Substance-specific informational needs that if met would reduce the uncertainties of human health assessment.

Developmental Toxicity—The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Dose-Response Relationship—The quantitative relationship between the amount of exposure to a toxicant and the incidence of the adverse effects.

Embryotoxicity and Fetotoxicity—Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the insult occurs. The terms, as used here, include malformations and variations, altered growth, and *in utero* death.

Environmental Protection Agency (EPA) Health Advisory—An estimate of acceptable drinking water levels for a chemical substance based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

Epidemiology—Refers to the investigation of factors that determine the frequency and distribution of disease or other health-related conditions within a defined human population during a specified period.

Genotoxicity—A specific adverse effect on the genome of living cells that, upon the duplication of affected cells, can be expressed as a mutagenic, clastogenic, or carcinogenic event because of specific alteration of the molecular structure of the genome.

Half-life—A measure of rate for the time required to eliminate one half of a quantity of a chemical from the body or environmental media.

Immediately Dangerous to Life or Health (IDLH)—The maximum environmental concentration of a contaminant from which one could escape within 30 minutes without any escape-impairing symptoms or irreversible health effects.

Immunologic Toxicity—The occurrence of adverse effects on the immune system that may result from exposure to environmental agents such as chemicals.

Immunological Effects—Functional changes in the immune response.

Incidence—The ratio of individuals in a population who develop a specified condition to the total number of individuals in that population who could have developed that condition in a specified time period.

Intermediate Exposure—Exposure to a chemical for a duration of 15–364 days, as specified in the Toxicological Profiles.

***In Vitro*—**Isolated from the living organism and artificially maintained, as in a test tube.

***In Vivo*—**Occurring within the living organism.

Lethal Concentration$_{(LO)}$ (LC_{LO})—The lowest concentration of a chemical in air that has been reported to have caused death in humans or animals.

Lethal Concentration$_{(50)}$ (LC_{50})—A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose$_{(LO)}$ (LD_{Lo})—The lowest dose of a chemical introduced by a route other than inhalation that has been reported to have caused death in humans or animals.

Lethal Dose$_{(50)}$ (LD_{50})—The dose of a chemical that has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time$_{(50)}$ (LT_{50})—A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL)—The lowest exposure level of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Lymphoreticular Effects—Represent morphological effects involving lymphatic tissues such as the lymph nodes, spleen, and thymus.

Malformations—Permanent structural changes that may adversely affect survival, development, or function.

Minimal Risk Level (MRL)—An estimate of daily human exposure to a hazardous substance that is likely to be without an appreciable risk of adverse noncancer health effects over a specified route and duration of exposure.

Modifying Factor (MF)—A value (greater than zero) that is applied to the derivation of a Minimal Risk Level (MRL) to reflect additional concerns about the database that are not covered by the uncertainty factors. The default value for a MF is 1.

Morbidity—State of being diseased; morbidity rate is the incidence or prevalence of disease in a specific population.

Mortality—Death; mortality rate is a measure of the number of deaths in a population during a specified interval of time.

Mutagen—A substance that causes mutations. A mutation is a change in the DNA sequence of a cell's DNA. Mutations can lead to birth defects, miscarriages, or cancer.

Necropsy—The gross examination of the organs and tissues of a dead body to determine the cause of death or pathological conditions.

Neurotoxicity—The occurrence of adverse effects on the nervous system following exposure to a chemical.

No-Observed-Adverse-Effect Level (NOAEL)—The dose of a chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Effects may be produced at this dose, but they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{ow})—The equilibrium ratio of the concentrations of a chemical in *n*-octanol and water, in dilute solution.

Odds Ratio (OR)—A means of measuring the association between an exposure (such as toxic substances and a disease or condition) that represents the best estimate of relative risk (risk as a ratio of the incidence among subjects exposed to a particular risk factor divided by the incidence among subjects who were not exposed to the risk factor). An OR of greater than 1 is considered to indicate greater risk of disease in the exposed group compared to the unexposed group.

Organophosphate or Organophosphorus Compound—A phosphorus-containing organic compound and especially a pesticide that acts by inhibiting cholinesterase.

Permissible Exposure Limit (PEL)—An Occupational Safety and Health Administration (OSHA) allowable exposure level in workplace air averaged over an 8-hour shift of a 40-hour workweek.

Pesticide—General classification of chemicals specifically developed and produced for use in the control of agricultural and public health pests.

Pharmacokinetics—The dynamic behavior of a material in the body, used to predict the fate (disposition) of an exogenous substance in an organism. Utilizing computational techniques, it provides the means of studying the absorption, distribution, metabolism, and excretion of chemicals by the body.

Pharmacokinetic Model—A set of equations that can be used to describe the time course of a parent chemical or metabolite in an animal system. There are two types of pharmacokinetic models: data-based and physiologically-based. A data-based model divides the animal system into a series of compartments, which, in general, do not represent real, identifiable anatomic regions of the body, whereas the physiologically-based model compartments represent real anatomic regions of the body.

Physiologically Based Pharmacodynamic (PBPD) Model—A type of physiologically based dose-response model that quantitatively describes the relationship between target tissue dose and toxic end points. These models advance the importance of physiologically based models in that they clearly describe the biological effect (response) produced by the system following exposure to an exogenous substance.

Physiologically Based Pharmacokinetic (PBPK) Model—Comprised of a series of compartments representing organs or tissue groups with realistic weights and blood flows. These models require a variety of physiological information: tissue volumes, blood flow rates to tissues, cardiac output, alveolar ventilation rates, and possibly membrane permeabilities. The models also utilize biochemical information, such as air/blood partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

Prevalence—The number of cases of a disease or condition in a population at one point in time.

Prospective Study—A type of cohort study in which the pertinent observations are made on events occurring after the start of the study. A group is followed over time.

q_1*—The upper-bound estimate of the low-dose slope of the dose-response curve as determined by the multistage procedure. The q_1* can be used to calculate an estimate of carcinogenic potency, the incremental excess cancer risk per unit of exposure (usually μg/L for water, mg/kg/day for food, and μg/m^3 for air).

Recommended Exposure Limit (REL)—A National Institute for Occupational Safety and Health (NIOSH) time-weighted average (TWA) concentration for up to a 10-hour workday during a 40-hour workweek.

Reference Concentration (RfC)—An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer health effects during a lifetime. The inhalation reference concentration is for continuous inhalation exposures and is appropriately expressed in units of mg/m^3 or ppm.

Reference Dose (RfD)—An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure of the human population to a potential hazard that is likely to be without risk of deleterious effects during a lifetime. The RfD is operationally derived from the no-observed-adverse-effect level (NOAEL, from animal and human studies) by a consistent application of uncertainty factors that reflect various types of data used to estimate RfDs and an additional modifying factor, which is based on a professional judgment of the entire database on the chemical. The RfDs are not applicable to nonthreshold effects such as cancer.

Reportable Quantity (RQ)—The quantity of a hazardous substance that is considered reportable under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Reportable quantities are (1) 1 pound or greater or (2) for selected substances, an amount established by regulation either under CERCLA or under Section 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

Reproductive Toxicity—The occurrence of adverse effects on the reproductive system that may result from exposure to a chemical. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

Retrospective Study—A type of cohort study based on a group of persons known to have been exposed at some time in the past. Data are collected from routinely recorded events, up to the time the study is undertaken. Retrospective studies are limited to causal factors that can be ascertained from existing records and/or examining survivors of the cohort.

Risk—The possibility or chance that some adverse effect will result from a given exposure to a chemical.

Risk Factor—An aspect of personal behavior or lifestyle, an environmental exposure, or an inborn or inherited characteristic that is associated with an increased occurrence of disease or other health-related event or condition.

Risk Ratio—The ratio of the risk among persons with specific risk factors compared to the risk among persons without risk factors. A risk ratio greater than 1 indicates greater risk of disease in the exposed group compared to the unexposed group.

Short-Term Exposure Limit (STEL)—The American Conference of Governmental Industrial Hygienists (ACGIH) maximum concentration to which workers can be exposed for up to 15 minutes continually. No more than four excursions are allowed per day, and there must be at least 60 minutes between exposure periods. The daily Threshold Limit Value-Time Weighted Average (TLV-TWA) may not be exceeded.

Standardized Mortality Ratio (SMR)—A ratio of the observed number of deaths and the expected number of deaths in a specific standard population.

Target Organ Toxicity—This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen—A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV)—An American Conference of Governmental Industrial Hygienists (ACGIH) concentration of a substance to which most workers can be exposed without adverse effect. The TLV may be expressed as a Time Weighted Average (TWA), as a Short-Term Exposure Limit (STEL), or as a ceiling limit (CL).

Time-Weighted Average (TWA)—An allowable exposure concentration averaged over a normal 8-hour workday or 40-hour workweek.

Toxic Dose$_{(50)}$ (TD$_{50}$)—A calculated dose of a chemical, introduced by a route other than inhalation, which is expected to cause a specific toxic effect in 50% of a defined experimental animal population.

Toxicokinetic—The absorption, distribution, and elimination of toxic compounds in the living organism.

Uncertainty Factor (UF)—A factor used in operationally deriving the Minimal Risk Level (MRL) or Reference Dose (RfD) or Reference Concentration (RfC) from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using lowest-observed-adverse-effect level (LOAEL) data rather than no-observed-adverse-effect level (NOAEL) data. A default for each individual UF is 10; if complete certainty in data exists, a value of 1 can be used; however, a reduced UF of 3 may be used on a case-by-case basis, 3 being the approximate logarithmic average of 10 and 1.

Xenobiotic—Any chemical that is foreign to the biological system.

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APPENDIX A. ATSDR MINIMAL RISK LEVELS AND WORKSHEETS

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) [42 U.S.C. 9601 et seq.], as amended by the Superfund Amendments and Reauthorization Act (SARA) [Pub. L. 99–499], requires that the Agency for Toxic Substances and Disease Registry (ATSDR) develop jointly with the U.S. Environmental Protection Agency (EPA), in order of priority, a list of hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL); prepare toxicological profiles for each substance included on the priority list of hazardous substances; and assure the initiation of a research program to fill identified data needs associated with the substances.

The toxicological profiles include an examination, summary, and interpretation of available toxicological information and epidemiologic evaluations of a hazardous substance. During the development of toxicological profiles, Minimal Risk Levels (MRLs) are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. MRLs are based on noncancer health effects only and are not based on a consideration of cancer effects. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the no-observed-adverse-effect level/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (365 days and longer) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive chemical-induced end point considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals.

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology and Environmental Medicine, expert panel peer reviews, and agency-wide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published levels. For additional information regarding MRLs, please contact the Division of Toxicology and Environmental Medicine, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop F-32, Atlanta, Georgia 30333.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Inorganic Arsenic
CAS Number: 7440-38-2
Date: August 2007
Profile Status: Post-Public Comment, Final Draft
Route: [ ] Inhalation [X] Oral
Duration: [X] Acute [ ] Intermediate [ ] Chronic
Graph Key: 29
Species: Human

Minimal Risk Level: 0.005 [X] mg/kg/day [ ] ppm

Reference: Mizuta N, Mizuta M, Ito F, et al. 1956. An outbreak of acute arsenic poisoning caused by arsenic-contaminated soy-sauce (shōyu): A clinical report of 220 cases. Bull Yamaguchi Med Sch 4(2-3):131-149.

Experimental design: Mizuta et al. (1956) summarized findings from 220 poisoning cases associated with an episode of arsenic contamination of soy sauce in Japan. The soy sauce was contaminated with approximately 0.1 mg As/mL, probably as calcium arsenate. Arsenic intake in the cases was estimated by the researchers to be 3 mg/day (0.05 mg/kg/day, assuming 55 kg average body weight for this Asian population). Duration of exposure was 2–3 weeks in most cases. Clinical symptoms were recorded. Seventy patients were examined opthalmologically. Laboratory tests were performed on some patients and included hematology, urinalysis, fecal exam, occult blood in gastric and duodenal juice, biochemical examination of blood, liver function tests, electrocardiograph, and liver biopsy.

Effects noted in study and corresponding doses: The primary symptoms were edema of the face, and gastrointestinal and upper respiratory symptoms initially, followed in some patients by skin lesions and neuropathy. Other effects included mild anemia and leukopenia, mild degenerative liver lesions and hepatic dysfunction, abnormal electrocardiogram, and ocular lesions. For derivation of the acute oral MRL, facial edema and gastrointestinal symptoms (nausea, vomiting, diarrhea), which were characteristic of the initial poisoning and then subsided, were considered to be the critical effects.

Dose and end point used for MRL derivation: 0.05 mg As/kg/day

[ ] NOAEL [X] LOAEL

Uncertainty factors used in MRL derivation:

[ ] 1 [ ] 3 [X] 10 (for use of a LOAEL)
[ ] 1 [ ] 3 [ ] 10 (for extrapolation from animals to humans)
[X] 1 [ ] 3 [ ] 10 (for human variability)

Was a conversion factor used from ppm in food or water to a mg/body weight dose? Not applicable.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? Not applicable.

Other additional studies or pertinent information that lend support to this MRL: The MRL is supported by the case of a man and wife in upstate New York who experienced gastrointestinal symptoms (nausea, diarrhea, abdominal cramps) starting almost immediately after beginning intermittent consumption of arsenic-tainted drinking water at an estimated dose of 0.05 mg As/kg/day (Franzblau and Lilis 1989). Gastrointestinal symptoms have been widely reported in other acute arsenic poisoning reports as well, although in some cases, the doses were higher and effects were severe, and in other cases, dose information was not available. The UF of 1 for intrahuman variability reflects the fact that the database includes persons of various ethnicities and age groups, including infants.

Agency Contact (Chemical Manager): Selene Chou, Ph.D and Carolyn Harper, Ph.D.

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Inorganic Arsenic
CAS Number: 7440-38-2
Date: August 2007
Profile Status: Post-Public Comment, Final Draft
Route: [ ] Inhalation [X] Oral
Duration: [ ] Acute [ ] Intermediate [X] Chronic
Graph Key: 134
Species: Human

Minimal Risk Level: 0.0003 [X] mg/kg/day [ ] ppm

References: Tseng, WP, Chu HM, How SW, et al. 1968. Prevalence of skin cancer in an endemic area of chronic arsenicism in Taiwan. J Natl Cancer Inst 40:453-463.

Tseng, WP. 1977. Effects and dose-response relationships of cancer and Blackfoot disease with arsenic. Environ Health Perspect 19:109-119.

Experimental design: Tseng et al. (1968) and Tseng (1977) investigated the incidence of Blackfoot disease and dermal lesions (hyperkeratosis and hyperpigmentation) in a large number of poor farmers (both male and female) exposed to high levels of arsenic in well water in Taiwan. A control group consisting of 17,000 people was identified. The authors stated that the incidence of dermal lesions increased with dose, but individual doses were not provided. However, incidence data were provided based on stratification of the exposed population into low (<300 μg/L), medium (300–600 μg/L), or high (>600 μg/L) exposure levels. Doses were calculated from group mean arsenic concentrations in well water, assuming the intake parameters described by Abernathy et al. (1989). Accordingly, the control, low-, medium-, and high-exposure levels correspond to doses of 0.0008, 0.014, 0.038, and 0.065 mg As/kg/day, respectively. The NOAEL identified by Tseng (1977) (0.0008 mg As/kg/day) was limited by the fact that the majority of the population was <20 years of age and the incidence of skin lesions increased as a function of age, and because the estimates of water intake and dietary arsenic intake are highly uncertain. Schoof et al. (1998) estimated that dietary intakes of arsenic from rice and yams may have been 15–211 μg/day (mean 61 μg/day), based on arsenic analyses of foods collected in Taiwan in 1993–1995. Use of the 50 μg/day estimate would result in an approximate doubling of the NOAEL (0.0016 mg/kg/day).

Effects noted in study and corresponding doses: A clear dose-response relationship was observed for characteristic skin lesions:

0.0008 mg As/kg/day	= control group (NOAEL)
0.014 mg As/kg/day	= hyperpigmentation and keratosis of the skin (less serious LOAEL)
0.038–0.065 mg As/kg/day	= increased incidence of dermal lesions

Dose and end point used for MRL derivation: 0.0008 mg As/kg/day

[X] NOAEL [ ] LOAEL

Uncertainty factors used in MRL derivation:

[ ] 1 [ ] 3 [ ] 10 (for use of a LOAEL)
[ ] 1 [ ] 3 [ ] 10 (for extrapolation from animals to humans)

[ ] 1 [x] 3 [ ] 10 (for human variability)

Was a conversion factor used from ppm in food or water to a mg/body weight dose? The arithmetic mean concentration of arsenic in well water for the control group (0.009 mg/L) was converted to a NOAEL of 0.0008 mg As/kg/day as described below:

$$\left[\left(\frac{0.009mg}{L}\times\frac{4.5L}{day}\right)+\frac{0.002mg}{day}\right]\div 55kg = 0.0008mgAs/kg/day$$

This NOAEL conversion assumed a water intake of 4.5 L/day and a body weight of 55 kg, and includes an estimation of arsenic intake of 0.002 mg As/kg/day from food. These assumptions are detailed in Abernathy et al. (1989). This approach to deriving a chronic oral MRL is identical to EPA's approach to deriving a chronic oral RfD.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? Not applicable.

Other additional studies or pertinent information that lend support to this MRL: The MRL is supported by a number of well conducted epidemiological studies that identify reliable NOAELs and LOAELs for dermal effects. EPA (1981b) identified a NOAEL of 0.006–0.007 mg As/kg/day for dermal lesions in several small populations in Utah. Harrington et al. (1978) identified a NOAEL of 0.003 mg As/kg/day for dermal effects in a small population in Alaska. Guha Mazumder et al. (1988) identified a NOAEL of 0.009 mg As/kg/day and a LOAEL of 0.006 mg As/kg/day for pigmentation changes and hyperkeratosis in a small population in India. Haque et al. (2003) identified a LOAEL of 0.0043 mg As/kg/day for hyperpigmentation and hyperkeratosis in a case-control study in India. Cebrían et al. (1983) identified a NOAEL of 0.0004 mg As/kg/day and a LOAEL of 0.022 mg As/kg/day in two regions in Mexico. Borgoño and Greiber (1972) and Zaldívar (1974) identified a LOAEL of 0.02 mg As/kg/day for abnormal skin pigmentation in patients in Chile, and Borgoño et al. (1980) identified a LOAEL of 0.01 mg As/kg/day for the same effect in school children in Chile. Valentine et al. (1985) reported a NOAEL of 0.02 mg As/kg/day for dermal effects in several small populations in California. Collectively, these studies indicate that the threshold dose for hyperpigmentation and hyperkeratosis is approximately 0.002 mg As/kg/day.

Agency Contacts (Chemical Managers): Selene Chou, Ph.D and Carolyn Harper, Ph.D.

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Monomethylarsonic acid (MMA)
CAS Number: 124-58-3
Date: August 2007
Profile Status: Post-Public Comment, Final Draft
Route: [ ] Inhalation [X] Oral
Duration: [ ] Acute [X] Intermediate [ ] Chronic
Graph Key: 12
Species: Rat

Minimal Risk Level: 0.1 [X] mg MMA/kg/day [ ] ppm

References: Arnold LL, Eldan M, van Gemert M, et al. 2003. Chronic studies evaluating the carcinogenicity of monomethylarsonic acid in rats and mice. Toxicology 190:197-219.

Crown S, Nyska A, Waner T. 1990. Methanearsonic acid: Combined chronic feeding and oncogenicity study in the rat. Conducted by Life Science Research Israel Ltd., Ness Ziona Israel. Submitted to EPA Office of Pesticide Programs (MRID 41669001).

Experimental design: Groups of 60 male and 60 female Fischer 344 rats were exposed to 0, 50, 400, or 1,300 ppm MMA in the diet for 104 weeks. Using the average doses for weeks 1–50 reported in an unpublished version of this study (Crown et al. 1990), doses of 0, 3.5, 30.2, and 106.9 mg MMA/kg/day and 0, 4.2, 35.9, and 123.3 mg MMA/kg/day were calculated for males and females, respectively. Body weights, food consumption, and water intake were monitored regularly. Blood was taken at 3, 6, and 12 months for clinical chemistry measurements, and urine samples were collected at the same interval.

Effects noted in study and corresponding doses: Mortality was increased in high-dose males and females during the first 52 weeks of the study. Body weights were decreased in the mid- and high-dose groups of both sexes; however, at 51 weeks, only the body weight for the high-dose males was <10% of the control weight (14.5%). Food and water consumption was increased in the mid- and high-dose groups. Diarrhea was observed in 100% of the high-dose males and females and in 16.7 and 40% of the mid-dose males and females during the first 52 weeks of exposure. Diarrhea first occurred after 3 weeks of exposure to the high dose and 4 weeks of exposure to the mid-dose group; the severity of the diarrhea was dose-related. The gastrointestinal system was the primary target in animals dying early; numerous macroscopic and histological alterations were observed.

Dose and end point used for MRL derivation: Benchmark dose analysis of the dose-response data (Table A-1) for diarrhea in male and female rats exposed to MMA in the diet for 1–52 weeks (incidence data reported in Crown et al. 1990) was conducted. All available dichotomous models in EPA's Benchmark Dose Software (version 1.4.1) were fit to the data. Predicted doses associated with a 10% extra risk were calculated. As assessed by the chi-square goodness-of-fit statistic, all models, with the exception of the quantal linear model for male incidence data and the quantal linear model for female incidence data, provided an adequate fit (X^2 p>0.1) (Table A-2). Comparing across models, a better fit is generally indicated by a lower Akaike's Information Criteria (AIC). As assessed by AIC, the gamma model for the males (Figure A-1) and the 2-degree polynomial multi-stage model for the females (Figure A-2) provide the best fit to the data. The predicted BMD_{10} and $BMDL_{10}$ are 28.25 mg MMA/kg/day and 22.99 mg MMA/kg/day for the male rat incidence data and 16.17 mg MMA/kg/day, and 12.38 mg MMA/kg/day for the female rat incidence data.

Table A-1. Incidences of Diarrhea in Rats Exposed to MMA in the Diet for 1–52 Weeks

Dietary concentration (ppm)	Dose (mg MMA/kg/day)	Incidence
Male rats		
0	0	2/60
50	3.5	0/60
400	30.2	10/60
1,300	106.9	60/60
Female rats		
0	0	0/60
50	4.2	0/60
400	35.9	24/60
1,300	123.3	60/60

Sources: Arnold et al. 2003; Crown et al. 1990

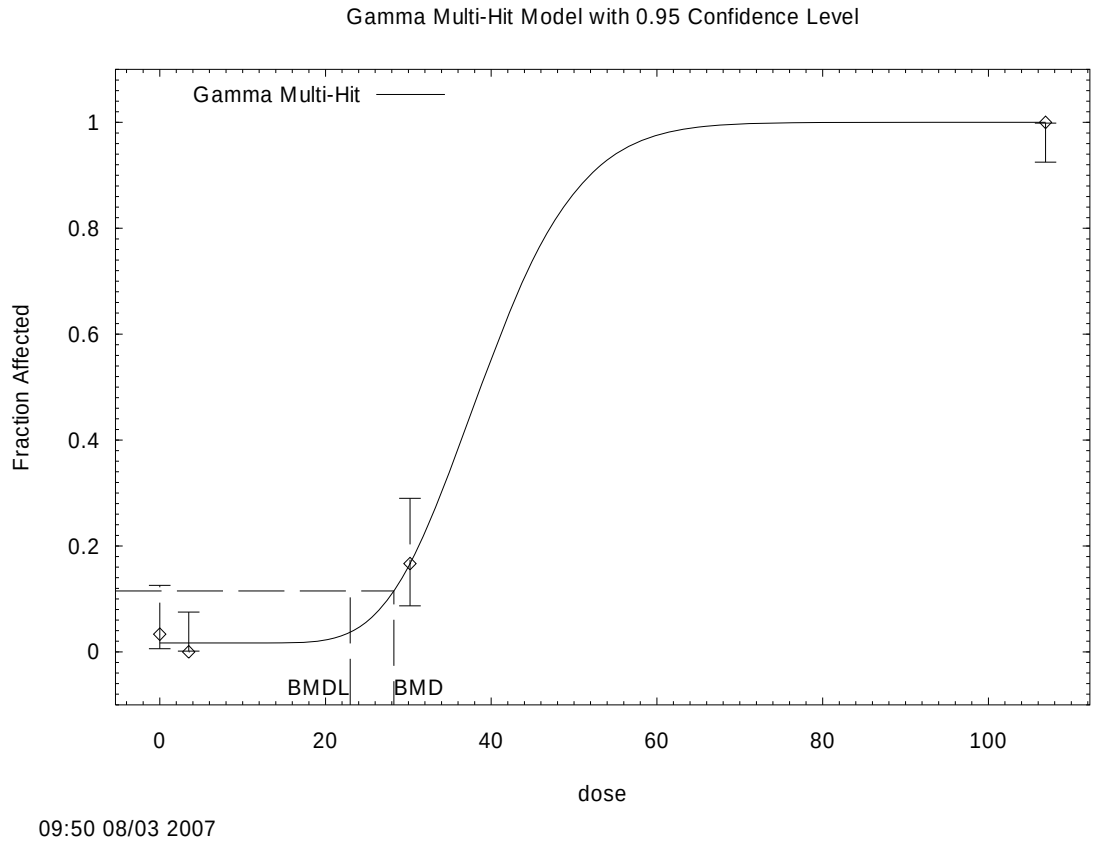
Table A-2. Modeling Predictions for the Incidence of Diarrhea in Rats Exposed to MMA in the Diet for 1–52 Weeks

Model	BMD_{10} (mg MMA/kg/day)	$BMDL_{10}$ (mg MMA/kg/day)	x^2 *p*-value	AIC
Male rats				
Gamma[a]	**28.25**	**22.99**	**0.36**	**78.41**
Logistic	24.60	20.19	0.16	79.59
Log-logistic[b]	29.32	24.73	0.15	80.41
Multi-stage[c]	25.74	19.90	0.35	78.51
Probit	23.11	18.67	0.11	80.02
Log-probit[b]	28.79	24.47	0.15	80.41
Quantal linear	6.317	5.079	0.00	123.06
Weibull[a]	27.99	20.66	0.15	80.41
Female rats				
Gamma[a]	26.81	15.18	1.00	84.76
Logistic	32.85	21.49	1.00	84.76
Log-logistic[b]	31.97	20.16	1.00	84.76
Multi-stage[c]	**16.17**	**12.38**	**0.90**	**83.88**
Probit	29.89	19.11	1.00	84.76
Log-probit[b]	28.95	18.87	1.00	84.76
Quantal linear	5.33	4.33	0.00	106.52
Weibull[a]	27.83	13.58	1.00	84.76

Sources: Arnold et al. 2003; Crown et al. 1990

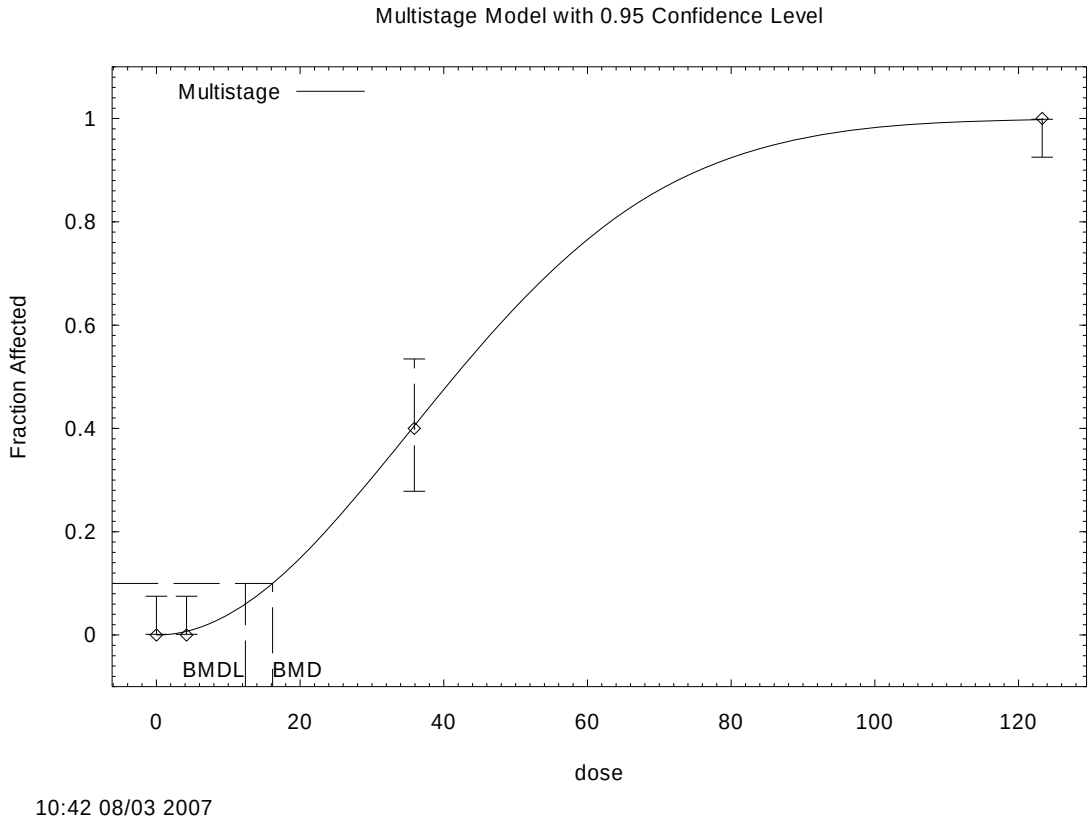
[a]Restrict power ≥1.
[b]Slope restricted to >1.
[c]Restrict betas ≥0; lowest degree polynomial with an adequate fit is reported; degree of polynomial=3.
[d]Restrict betas ≥0; lowest degree polynomial with an adequate fit is reported; degree of polynomial=2.

Figure A-1. Predicted and Observed Incidence of Diarrhea in Male Rats Exposed to MMA in the Diet for 1–52 Weeks*



*BMDs and BMDLs indicated are associated with a 10% extra risk change from the control, and are in units of mg MMA/kg/day.

Figure A-2. Predicted and Observed Incidence of Diarrhea in Female Rats Exposed to MMA in the Diet for 52 Weeks*



*BMDs and BMDLs indicated are associated with a 10% extra risk change from the control, and are in units of mg MMA/kg/day.

The $BMDL_{10}$ of 12.38 mg MMA/kg/day for female rats was selected as the point of departure for deriving the intermediate-duration oral MRL because it was lower than the $BMDL_{10}$ (22.99 mg MMA/kg/day) calculated using the male incidence data.

[ ] NOAEL [ ] LOAEL [X] BMDL

Uncertainty factors used in MRL derivation: 100

[ ] 10 for use of a LOAEL
[X] 10 for extrapolation from animals to humans
[X] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose? Doses calculated using the average of the achieved doses for weeks 1–50 reported in Crown et al. (1990): 0, 3.5, 30.2, and 106.9 mg MMA/kg/day for males and 0, 4.2, 35.9, and 123.3 mg MMA/kg/day for females.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? Not applicable.

Other additional studies or pertinent information that lend support to this MRL: Increases in the incidence of diarrhea has also been observed in dogs administered via capsule 2 mg MMA/kg/day for 52 weeks (Waner and Nyska 1988); the increased incidence of diarrhea started during weeks 25–28. At 35 mg MMA/kg/day, vomiting was also observed in the dogs. Diarrhea has also been observed in rats and mice exposed to MMA for 2 years (Arnold et al. 2003); the LOAELs are 25.7 and 67.1 mg MMA/kg/day, respectively.

Agency Contacts (Chemical Managers): Selene Chou, Ph.D and Carolyn Harper, Ph.D.

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Monomethylarsonic acid (MMA)
CAS Number: 124-58-3
Date: August 2007
Profile Status: Post-Public Comment, Final Draft
Route: [ ] Inhalation [X] Oral
Duration: [ ] Acute [ ] Intermediate [X] Chronic
Graph Key: 21
Species: Mouse

Minimal Risk Level: 0.01 [X] mg MMA/kg/day [ ] ppm

References: Arnold LL, Eldan M, van Gemert M, et al. 2003. Chronic studies evaluating the carcinogenicity of monomethylarsonic acid in rats and mice. Toxicology 190:197-219.

Gur E, Piraic H, Waner T. 1991. Methanearsonic acid: Combined oncogenicity study in the mouse. Conducted by Life Science Research Israel Ltd., Ness Ziona Israel. Submitted to EPA Office of Pesticide Programs (MRID 42173201).

Experimental design: Groups of 52 male and 52 female $B6C3F_1$ mice were exposed to 0, 10, 50, 200, or 400 ppm of MMA in the diet for 104 weeks. The reported MMA doses were 0, 1.2, 6.0, 24.9, and 67.1 mg MMA/kg/day (males) and 0, 1.4, 7.0, 31.2, and 101 mg MMA/kg/day (females). Body weights, food consumption, and water intake were monitored regularly. Blood was taken at 3, 6, 12, 18, and 24 months for white cell counts. At sacrifice, complete necropsies were performed, including histological examination of at least 13 organs.

Effects noted in study and corresponding doses: No treatment-related increases in mortality were observed. Significant decreases in body weights were observed in males and females exposed to 32.2 or 48.5 mg As/kg/day, respectively; at week 104, the males weighed 17% less than controls and females weighed 23% less. Food consumption was increased in females exposed to 101 mg MMA/kg/day, and water consumption was increased in 67.1 mg MMA/kg/day males and 31.2 and 101 mg MMA/kg/day females. Loose and mucoid feces were noted in mice exposed to 67.1/101 mg MMA/kg/day. No changes were seen in white cell counts of either sex. Small decreases in the weights of heart, spleen, kidney, and liver were seen in some animals, but the decreases were not statistically significant. Squamous metaplasia of the cecum, colon, and rectum was observed at 67.1/101 mg MMA/kg/day. The incidences of metaplasia in the cecum, colon, and rectum were 29/49, 14/49, and 39/49 in males and 38/52, 17/52, and 42/52 in females; metaplasia was not observed in other groups of male or female mice. An increased incidence of progressive glomerulonephropathy (incidence of 25/52, 27/52, 38/52, 39/52, and 46/52 in the 0, 1.2, 6.0, 24.9, and 67.1 mg MMA/kg/day) was observed in males; the incidence was significantly higher (Fisher Exact Test) than controls at ≥6.0 mg MMA/kg/day. Significant increases in the incidence of nephrocalcinosis was observed in the males at 24.9 and 67.1 mg MMA/kg/day (Fisher Exact Test) (incidence of 25/52, 30/52, 30/52, 45/522 45/51 and 0/52, 1/52, 1/52, 2/52, and 5/52 in males and females, respectively). The investigators noted that the kidney lesions were consistent with the normal spectrum of spontaneous renal lesions and there was no difference in character or severity of the lesions between groups. A reduction in the incidence of cortical focal hyperplasia in the adrenal gland of male mice exposed to 67.1 mg MMA/kg/day was possibly related to MMA exposure; the toxicological significance of this effect is not known.

Dose and end point used for MRL derivation: Benchmark dose analysis of the dose-response data (Table A-3) for progressive glomerulonephropathy in male mice exposed to MMA in the diet for 2 years

(incidence data reported in Gur et al. 1991) was conducted. All available dichotomous models in EPA's Benchmark Dose Software (version 1.4.1) were fit to the data. Predicted doses associated with a 10% extra risk were calculated. As assessed by the chi-square goodness-of-fit statistic, all models, with the exception of the log-probit model, provided an adequate fit (X^2 p>0.1) (Table A-4). Comparing across models, a better fit is generally indicated by a lower Akaike's Information Criteria (AIC). As assessed by AIC, the log-logistic model (Figure A-3) provided the best fit to the data. The predicted BMD_{10} and $BMDL_{10}$ for the incidence data are 2.09 and 1.09 mg MMA/kg/day.

Table A-3. Incidence of Progressive Glomerulonephropathy in Male Mice Exposed to MMA in the Diet for 2 Years

Dietary Concentration (ppm)	Dose (mg MMA/kg/day)	Incidence
0	0	25/52
10	1.2	27/52
50	6.0	38/52
200	24.9	39/52
400	67.1	46/52

Sources: Arnold et al. 2003; Gur et al. 1991

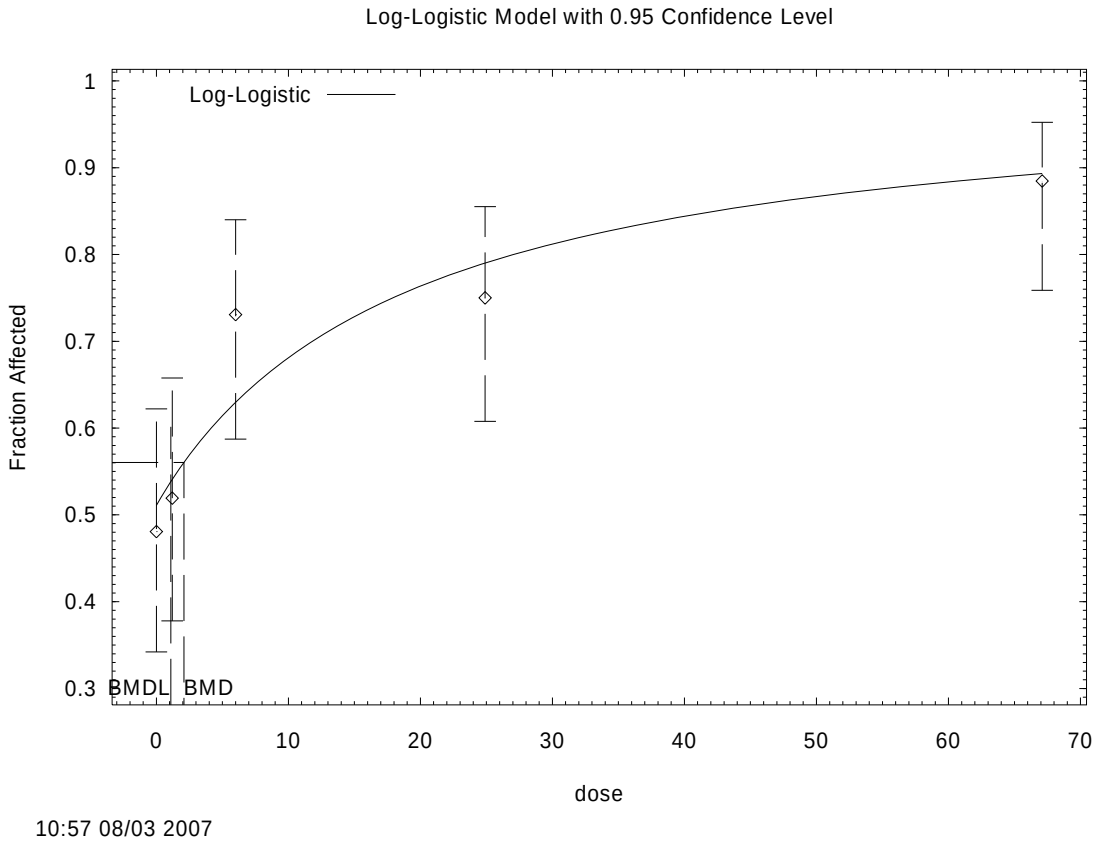
Table A-4. Modeling Predictions for the Incidence of Progressive Glomerulonephropathy in Male Mice Exposed to MMA in the Diet for 2 Years

Model	BMD_{10} (mg MMA/kg/day)	$BMDL_{10}$ (mg MMA/kg/day)	x^2 p-value	AIC
Gamma[a]	4.60	3.15	0.18	309.33
Logistic	6.09	4.45	0.13	310.15
Log-logistic[b]	**2.09**	**1.09**	**0.38**	**307.47**
Multi-stage[c]	4.60	3.15	0.18	309.33
Probit	6.62	5.00	0.11	310.43
Log-probit[b]	8.54	5.50	0.08	311.11
Quantal linear	4.60	3.15	0.18	309.33
Weibull[a]	4.60	3.15	0.18	309.33

Sources: Arnold et al. 2003; Gur et al. 1991

[a]Restrict power ≥1.
[b]Slope restricted to >1.
[c]Restrict betas ≥0; lowest degree polynomial with an adequate fit is reported; degree of polynomial=1.

Figure A-3. Predicted and Observed Incidence of Progressive Glomerulonephropathy in Male Mice Exposed to MMA*



*BMDs and BMDLs indicated are associated with a 10% extra risk change from the control, and are in units of mg MMA/kg/day.

The $BMDL_{10}$ of 1.09 mg MMA/kg/day for male mice was selected as the point of departure for deriving the chronic-duration oral MRL.

[ ] NOAEL [ ] LOAEL [X] BMDL

Uncertainty factors used in MRL derivation: 100

[ ] 10 for use of a LOAEL
[X] 10 for extrapolation from animals to humans
[X] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose? Doses calculated using the average of the achieved doses reported in Gur et al. (1991): 0, 1.2, 6.0, 24.9, and 67.1 mg MMA/kg/day for males and 0, 1.4, 7.0, 31.2, and 101 mg MMA/kg/day for females.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? Not applicable.

Other additional studies or pertinent information that lend support to this MRL: An exacerbation of chronic progressive nephropathy (an increase in the severity of the nephropathy) has also been observed in rats exposed to ≥33.9 mg MMA/kg/day for 2 years (Arnold et al. 2003).

Agency Contacts (Chemical Managers): Selene Chou, Ph.D and Carolyn Harper, Ph.D.

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Dimethylarsinic acid (DMA)
CAS Number: 75-60-5
Date: August 2007
Profile Status: Post-Public Comment, Final Draft
Route: [ ] Inhalation [X] Oral
Duration: [ ] Acute [ ] Intermediate [X] Chronic
Graph Key: 35
Species: Mouse

Minimal Risk Level: 0.02 [X] mg DMA/kg/day [ ] ppm

References: Arnold LL, Eldan M, Nyska A, et al. 2006. Dimethylarsinic acid: Results of chronic toxicity/oncogenicity studies in F344 rats and $B6C3F_1$ mice. Toxicology 223:82-100.

Gur E, Nyska A, Pirak M, et al. 1989b. Cacodylic acid: Oncogenicity study in the mouse. Conducted by Life Science Research Israel Ltd., Ness Ziona Israel. Submitted to EPA Office of Pesticide Programs (MRID 41914601).

Experimental design: Groups of 56 male and 56 female $B6C3F_1$ mice were exposed to 0, 8, 40, 200, or 500 ppm DMA in the diet for 2 years. The investigators reported the dietary doses were equivalent to approximately 0, 1.3, 7.8, 37, and 94 mg DMA/kg/day. The following parameters were used to assess toxicity: clinical observations, body weight, food consumption, water consumption, differential leukocyte levels measured at 12, 18, and 24 months in mice in the control and 94 mg DMA/kg/day groups, organ weights (brain, kidneys, liver, and testes), and histopathological examination of major tissues and organs.

Effects noted in study and corresponding doses: No deaths were observed. Decreases in body weight gain were observed in the male mice exposed to 94 mg DMA/kg/day; the difference was <10% and was not considered adverse. An increase in water consumption was observed in males exposed to 94 mg DMA/kg/day during weeks 60–96. No treatment-related clinical signs were observed. In the female mice exposed to 94 mg DMA/kg/day, a statistically significant decrease in lymphocytes and increase in monocytes were observed at 24 months. Treatment related nonneoplastic alterations were observed in the urinary bladder and kidneys. In the urinary bladder, increases in the vacuolization of the superficial cells of the urothelium were observed in males exposed to 37 or 94 mg DMA/kg/day (0/44, 1/50, 0/50, 36/45, 48/48) and in females exposed to 7.8, 37, or 94 mg DMA/kg/day (1/45, 1/48, 26/43, 47/47, 43/43); incidence data reported in Gur et al. (1989b). An increased incidence of progressive glomerulonephropathy was observed in males at 37 mg DMA/kg/day (16/44, 22/50, 17/50, 34/45, 30/50) and an increased incidence of nephrocalcinosis was also observed in male mice at 94 mg DMA/kg/day (30/44, 25/50, 27/50, 29/50, 45/50). Neoplastic alterations were limited to an increased incidence of fibrosarcoma of the skin in females exposed to 94 mg DMA/kg/day; the incidence was 3/56, 0/55, 1/56, 1/56, and 6/56 in the 0, 1.3, 7.8, 37, and 94 mg DMA/kg/day groups, respectively; however it was concluded that this lesion was not related to DMA exposure.

Dose and end point used for MRL derivation: Benchmark dose analysis of the dose-response data (Table A-5) for vacuolization of the urothelium in the urinary bladder in female mice exposed to DMA in the diet for 2 years (incidence data reported in Gur et al. 1989b) was conducted. All available dichotomous models in EPA's Benchmark Dose Software (version 1.4.1) were fit to the data. Predicted doses associated with a 10% extra risk were calculated. As assessed by the chi-square goodness-of-fit statistic, all models, with the exception of the quantal linear model, provided an adequate fit (X^2 p>0.1) (Table A-6). Comparing across models, a better fit is generally indicated by a lower AIC. The AIC

values were similar for the logistic, multi-stage, and probit models; of these three models, the multi-stage had the lowest BMD_{10} and was selected for the analysis (see Figure A-4). The predicted BMD_{10} and $BMDL_{10}$ for the incidence data are 2.68 and 1.80 mg DMA/kg/day.

Table A-5. Incidence of Vacuolization of Urotheium in Urinary Bladder of Female Mice Exposed to DMA in the Diet for 2 Years

Dietary concentration (ppm)	Dose (mg DMA/kg/day)	Incidence
0	0	1/45
8	1.3	1/48
40	7.8	26/43
200	37	47/47
500	94	43/43

Sources: Arnold et al. 2006; Gur et al. 1989b

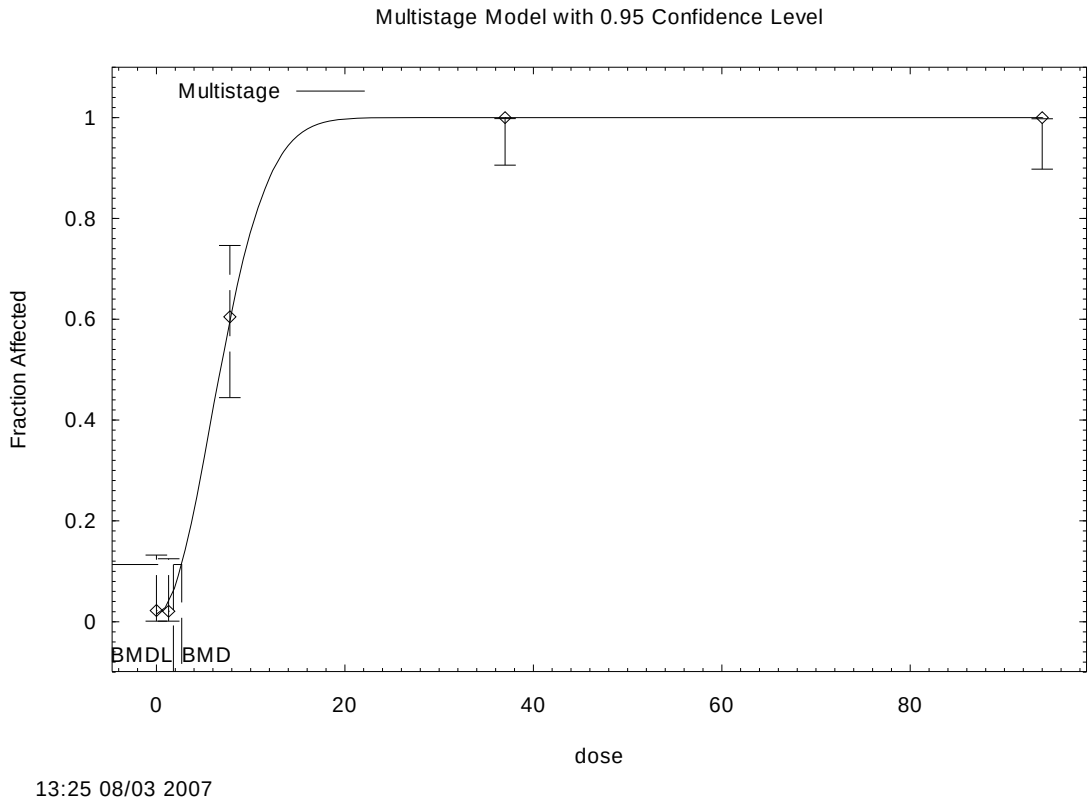
Table A-6. Modeling Predictions for the Incidence of Vacuolization in of Urothelium in Urinary Bladder of Female Mice Exposed to DMA in the Diet for 2 Years

Model	BMD_{10} (mg DMA/kg/day)	$BMDL_{10}$ (mg DMA/kg/day)	x^2 *p*-value	AIC
Gamma[a]	5.01	1.85	1.00	83.03
Logistic	3.66	2.78	0.95	81.37
Log-logistic[b]	6.23	2.34	1.00	83.03
Multi-stage[c]	**2.68**	**1.80**	**0.90**	**81.69**
Probit	3.20	2.46	0.89	81.60
Log-probit[b]	5.03	2.00	1.00	83.03
Quantal linear	0.98	0.76	0.07	91.75
Weibull[a]	4.77	1.88	1.00	83.03

Sources: Arnold et al. 2006; Gur et al. 1989b

[a]Restrict power ≥1.
[b]Slope restricted to >1.
[c]Restrict betas ≥0; lowest degree polynomial with an adequate fit is reported; degree of polynomial=2.

Figure A-4. Predicted and Observed Incidence of Vacuolization of Urothelium in Urinary Bladder of Female Mice*



Source: Arnold et al. 2006

*BMDs and BMDLs indicated are associated with a 10% extra risk change from the control, and are in units of mg DMA/kg/day.

The $BMDL_{10}$ of 1.80 mg DMA/kg/day for female mice was selected as the point of departure for deriving the chronic-duration oral MRL.

[ ] NOAEL [ ] LOAEL [X] BMDL

Uncertainty factors used in MRL derivation: 100

[ ] 10 for use of a LOAEL
[X] 10 for extrapolation from animals to humans
[X] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose? Doses reported in Gur et al. (1989b): 0, 1.3, 7.8, 37, and 94 mg DMA/kg/day.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? Not applicable.

Other additional studies or pertinent information that lend support to this MRL: One other study has investigated the chronic toxicity of DMA in species other than rats. In this study, administration of 16 mg DMA/kg/day via a capsule for 52 weeks resulted in increases in the incidence of diarrhea; no histological alterations were observed (Zomber et al. 1989).

Agency Contacts (Chemical Managers): Selene Chou, Ph.D and Carolyn Harper, Ph.D.

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APPENDIX B. USER'S GUIDE

Chapter 1

Public Health Statement

This chapter of the profile is a health effects summary written in nontechnical language. Its intended audience is the general public, especially people living in the vicinity of a hazardous waste site or chemical release. If the Public Health Statement were removed from the rest of the document, it would still communicate to the lay public essential information about the chemical.

The major headings in the Public Health Statement are useful to find specific topics of concern. The topics are written in a question and answer format. The answer to each question includes a sentence that will direct the reader to chapters in the profile that will provide more information on the given topic.

Chapter 2

Relevance to Public Health

This chapter provides a health effects summary based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information. This summary is designed to present interpretive, weight-of-evidence discussions for human health end points by addressing the following questions:

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

The chapter covers end points in the same order that they appear within the Discussion of Health Effects by Route of Exposure section, by route (inhalation, oral, and dermal) and within route by effect. Human data are presented first, then animal data. Both are organized by duration (acute, intermediate, chronic). *In vitro* data and data from parenteral routes (intramuscular, intravenous, subcutaneous, etc.) are also considered in this chapter.

The carcinogenic potential of the profiled substance is qualitatively evaluated, when appropriate, using existing toxicokinetic, genotoxic, and carcinogenic data. ATSDR does not currently assess cancer potency or perform cancer risk assessments. Minimal Risk Levels (MRLs) for noncancer end points (if derived) and the end points from which they were derived are indicated and discussed.

Limitations to existing scientific literature that prevent a satisfactory evaluation of the relevance to public health are identified in the Chapter 3 Data Needs section.

Interpretation of Minimal Risk Levels

Where sufficient toxicologic information is available, ATSDR has derived MRLs for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not

meant to support regulatory action, but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans.

MRLs should help physicians and public health officials determine the safety of a community living near a chemical emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Chapter 2, "Relevance to Public Health," contains basic information known about the substance. Other sections such as Chapter 3 Section 3.9, "Interactions with Other Substances," and Section 3.10, "Populations that are Unusually Susceptible" provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology that the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the most sensitive end point which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen end point are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest no-observed-adverse-effect level (NOAEL) that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor (UF) of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a substance-specific MRL are provided in the footnotes of the levels of significant exposure (LSE) tables.

Chapter 3

Health Effects

Tables and Figures for Levels of Significant Exposure (LSE)

Tables and figures are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species, MRLs to humans for noncancer end points, and EPA's estimated range associated with an upper- bound individual lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. Use the LSE tables and figures for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of NOAELs, LOAELs, or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE Table 3-1 and Figure 3-1 are shown. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

LEGEND

See Sample LSE Table 3-1 (page B-6)

(1) Route of Exposure. One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. Typically when sufficient data exist, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure, i.e., inhalation, oral, and dermal (LSE Tables 3-1, 3-2, and 3-3, respectively). LSE figures are limited to the inhalation (LSE Figure 3-1) and oral (LSE Figure 3-2) routes. Not all substances will have data on each route of exposure and will not, therefore, have all five of the tables and figures.

(2) Exposure Period. Three exposure periods—acute (less than 15 days), intermediate (15–364 days), and chronic (365 days or more)—are presented within each relevant route of exposure. In this example, an inhalation study of intermediate exposure duration is reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Health Effect. The major categories of health effects included in LSE tables and figures are death, systemic, immunological, neurological, developmental, reproductive, and cancer. NOAELs and LOAELs can be reported in the tables and figures for all effects but cancer. Systemic effects are further defined in the "System" column of the LSE table (see key number 18).

(4) Key to Figure. Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 18 has been used to derive a NOAEL and a Less Serious LOAEL (also see the two "18r" data points in sample Figure 3-1).

(5) Species. The test species, whether animal or human, are identified in this column. Chapter 2, "Relevance to Public Health," covers the relevance of animal data to human toxicity and Section 3.4, "Toxicokinetics," contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(6) Exposure Frequency/Duration. The duration of the study and the weekly and daily exposure regimens are provided in this column. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 18), rats were exposed to “Chemical x” via inhalation for 6 hours/day, 5 days/week, for 13 weeks. For a more complete review of the dosing regimen, refer to the appropriate sections of the text or the original reference paper (i.e., Nitschke et al. 1981).

(7) System. This column further defines the systemic effects. These systems include respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, and dermal/ocular. "Other" refers to any systemic effect (e.g., a decrease in body weight) not covered in these systems. In the example of key number 18, one systemic effect (respiratory) was investigated.

(8) NOAEL. A NOAEL is the highest exposure level at which no harmful effects were seen in the organ system studied. Key number 18 reports a NOAEL of 3 ppm for the respiratory system,

which was used to derive an intermediate exposure, inhalation MRL of 0.005 ppm (see footnote "b").

(9) LOAEL. A LOAEL is the lowest dose used in the study that caused a harmful health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific end point used to quantify the adverse effect accompanies the LOAEL. The respiratory effect reported in key number 18 (hyperplasia) is a Less Serious LOAEL of 10 ppm. MRLs are not derived from Serious LOAELs.

(10) Reference. The complete reference citation is given in Chapter 9 of the profile.

(11) CEL. A CEL is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases.

(12) Footnotes. Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. Footnote "b" indicates that the NOAEL of 3 ppm in key number 18 was used to derive an MRL of 0.005 ppm.

LEGEND

See Sample Figure 3-1 (page B-7)

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(13) Exposure Period. The same exposure periods appear as in the LSE table. In this example, health effects observed within the acute and intermediate exposure periods are illustrated.

(14) Health Effect. These are the categories of health effects for which reliable quantitative data exists. The same health effects appear in the LSE table.

(15) Levels of Exposure. Concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(16) NOAEL. In this example, the open circle designated 18r identifies a NOAEL critical end point in the rat upon which an intermediate inhalation exposure MRL is based. The key number 18 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 3 ppm (see entry 18 in the table) to the MRL of 0.005 ppm (see footnote "b" in the LSE table).

(17) CEL. Key number 38m is one of three studies for which CELs were derived. The diamond symbol refers to a CEL for the test species-mouse. The number 38 corresponds to the entry in the LSE table.

(18) Estimated Upper-Bound Human Cancer Risk Levels. This is the range associated with the upper-bound for lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. These risk levels are derived from the EPA's Human Health Assessment Group's upper-bound estimates of the slope of the cancer dose response curve at low dose levels (q_1*).

(19) Key to LSE Figure. The Key explains the abbreviations and symbols used in the figure.

SAMPLE

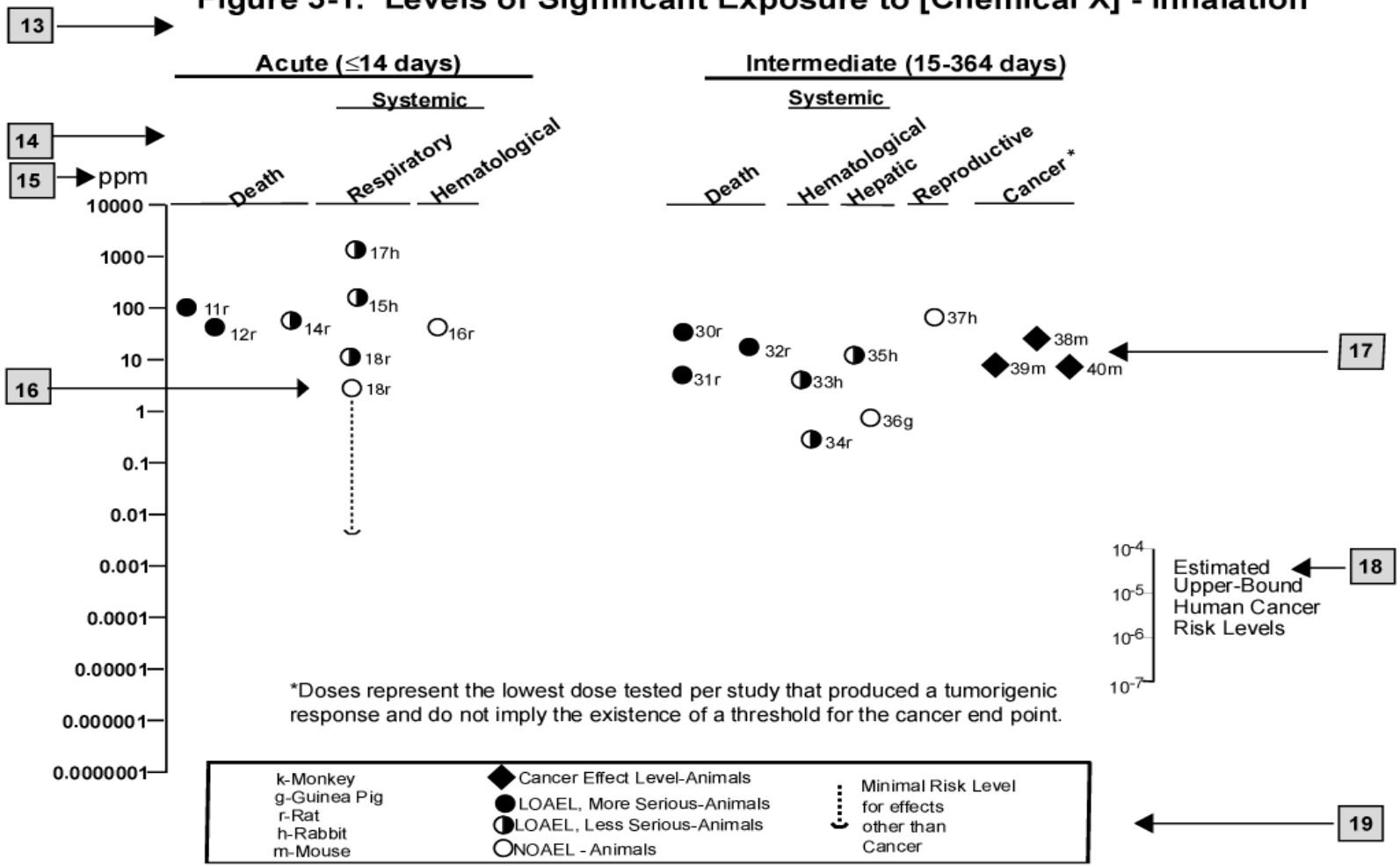
1 → **Table 3-1. Levels of Significant Exposure to [Chemical x] – Inhalation**

	Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
2 →	INTERMEDIATE EXPOSURE							
		5	6	7	8	9		10
3 →	Systemic	↓	↓	↓	↓	↓		↓
4 →	18	Rat	13 wk 5 d/wk 6 hr/d	Resp	3[b]	10 (hyperplasia)		Nitschke et al. 1981
	CHRONIC EXPOSURE							
	Cancer						11	
							↓	
	38	Rat	18 mo 5 d/wk 7 hr/d				20 (CEL, multiple organs)	Wong et al. 1982
	39	Rat	89–104 wk 5 d/wk 6 hr/d				10 (CEL, lung tumors, nasal tumors)	NTP 1982
	40	Mouse	79–103 wk 5 d/wk 6 hr/d				10 (CEL, lung tumors, hemangiosarcomas)	NTP 1982

12 → [a] The number corresponds to entries in Figure 3-1.
[b] Used to derive an intermediate inhalation Minimal Risk Level (MRL) of 5×10^{-3} ppm; dose adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animal to humans, 10 for human variability).

SAMPLE

Figure 3-1. Levels of Significant Exposure to [Chemical X] - Inhalation



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APPENDIX C. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
ACOEM	American College of Occupational and Environmental Medicine
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
AED	atomic emission detection
AFID	alkali flame ionization detector
AFOSH	Air Force Office of Safety and Health
ALT	alanine aminotransferase
AML	acute myeloid leukemia
AOAC	Association of Official Analytical Chemists
AOEC	Association of Occupational and Environmental Clinics
AP	alkaline phosphatase
APHA	American Public Health Association
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AWQC	Ambient Water Quality Criteria
BAT	best available technology
BCF	bioconcentration factor
BEI	Biological Exposure Index
BMD	benchmark dose
BMR	benchmark response
BSC	Board of Scientific Counselors
C	centigrade
CAA	Clean Air Act
CAG	Cancer Assessment Group of the U.S. Environmental Protection Agency
CAS	Chemical Abstract Services
CDC	Centers for Disease Control and Prevention
CEL	cancer effect level
CELDS	Computer-Environmental Legislative Data System
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
Ci	curie
CI	confidence interval
CL	ceiling limit value
CLP	Contract Laboratory Program
cm	centimeter
CML	chronic myeloid leukemia
CPSC	Consumer Products Safety Commission
CWA	Clean Water Act
DHEW	Department of Health, Education, and Welfare
DHHS	Department of Health and Human Services
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	Department of Energy
DOL	Department of Labor
DOT	Department of Transportation

DOT/UN/NA/IMCO	Department of Transportation/United Nations/North America/Intergovernmental Maritime Dangerous Goods Code
DWEL	drinking water exposure level
ECD	electron capture detection
ECG/EKG	electrocardiogram
EEG	electroencephalogram
EEGL	Emergency Exposure Guidance Level
EPA	Environmental Protection Agency
F	Fahrenheit
F_1	first-filial generation
FAO	Food and Agricultural Organization of the United Nations
FDA	Food and Drug Administration
FEMA	Federal Emergency Management Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FPD	flame photometric detection
fpm	feet per minute
FR	Federal Register
FSH	follicle stimulating hormone
g	gram
GC	gas chromatography
gd	gestational day
GLC	gas liquid chromatography
GPC	gel permeation chromatography
HPLC	high-performance liquid chromatography
HRGC	high resolution gas chromatography
HSDB	Hazardous Substance Data Bank
IARC	International Agency for Research on Cancer
IDLH	immediately dangerous to life and health
ILO	International Labor Organization
IRIS	Integrated Risk Information System
Kd	adsorption ratio
kg	kilogram
kkg	metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{50}	lethal concentration, 50% kill
LC_{Lo}	lethal concentration, low
LD_{50}	lethal dose, 50% kill
LD_{Lo}	lethal dose, low
LDH	lactic dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
LT_{50}	lethal time, 50% kill
m	meter
MA	*trans,trans*-muconic acid
MAL	maximum allowable level
mCi	millicurie
MCL	maximum contaminant level

MCLG	maximum contaminant level goal
MF	modifying factor
MFO	mixed function oxidase
mg	milligram
mL	milliliter
mm	millimeter
mmHg	millimeters of mercury
mmol	millimole
mppcf	millions of particles per cubic foot
MRL	Minimal Risk Level
MS	mass spectrometry
NAAQS	National Ambient Air Quality Standard
NAS	National Academy of Science
NATICH	National Air Toxics Information Clearinghouse
NATO	North Atlantic Treaty Organization
NCE	normochromatic erythrocytes
NCEH	National Center for Environmental Health
NCI	National Cancer Institute
ND	not detected
NFPA	National Fire Protection Association
ng	nanogram
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NIOSHTIC	NIOSH's Computerized Information Retrieval System
NLM	National Library of Medicine
nm	nanometer
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NOES	National Occupational Exposure Survey
NOHS	National Occupational Hazard Survey
NPD	nitrogen phosphorus detection
NPDES	National Pollutant Discharge Elimination System
NPL	National Priorities List
NR	not reported
NRC	National Research Council
NS	not specified
NSPS	New Source Performance Standards
NTIS	National Technical Information Service
NTP	National Toxicology Program
ODW	Office of Drinking Water, EPA
OERR	Office of Emergency and Remedial Response, EPA
OHM/TADS	Oil and Hazardous Materials/Technical Assistance Data System
OPP	Office of Pesticide Programs, EPA
OPPT	Office of Pollution Prevention and Toxics, EPA
OPPTS	Office of Prevention, Pesticides and Toxic Substances, EPA
OR	odds ratio
OSHA	Occupational Safety and Health Administration
OSW	Office of Solid Waste, EPA
OTS	Office of Toxic Substances
OW	Office of Water

OWRS	Office of Water Regulations and Standards, EPA
PAH	polycyclic aromatic hydrocarbon
PBPD	physiologically based pharmacodynamic
PBPK	physiologically based pharmacokinetic
PCE	polychromatic erythrocytes
PEL	permissible exposure limit
pg	picogram
PHS	Public Health Service
PID	photo ionization detector
pmol	picomole
PMR	proportionate mortality ratio
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
PSNS	pretreatment standards for new sources
RBC	red blood cell
REL	recommended exposure level/limit
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
RQ	reportable quantity
RTECS	Registry of Toxic Effects of Chemical Substances
SARA	Superfund Amendments and Reauthorization Act
SCE	sister chromatid exchange
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
SIC	standard industrial classification
SIM	selected ion monitoring
SMCL	secondary maximum contaminant level
SMR	standardized mortality ratio
SNARL	suggested no adverse response level
SPEGL	Short-Term Public Emergency Guidance Level
STEL	short term exposure limit
STORET	Storage and Retrieval
TD_{50}	toxic dose, 50% specific toxic effect
TLV	threshold limit value
TOC	total organic carbon
TPQ	threshold planning quantity
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TWA	time-weighted average
UF	uncertainty factor
U.S.	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey
VOC	volatile organic compound
WBC	white blood cell
WHO	World Health Organization

>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to
%	percent
α	alpha
β	beta
γ	gamma
δ	delta
μm	micrometer
μg	microgram
q_1^*	cancer slope factor
–	negative
+	positive
(+)	weakly positive result
(–)	weakly negative result

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TOXICOLOGICAL PROFILE FOR BENZENE

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

August 2007

DISCLAIMER

The use of company or product name(s) is for identification only and does not imply endorsement by the Agency for Toxic Substances and Disease Registry.

UPDATE STATEMENT

A Toxicological Profile for Benzene, Draft for Public Comment was released in August 2005. This edition supersedes any previously released draft or final profile.

Toxicological profiles are revised and republished as necessary. For information regarding the update status of previously released profiles, contact ATSDR at:

Agency for Toxic Substances and Disease Registry
Division of Toxicology and Environmental Medicine/Applied Toxicology Branch
1600 Clifton Road NE
Mailstop F-32
Atlanta, Georgia 30333

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FOREWORD

This toxicological profile is prepared in accordance with guidelines developed by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA). The original guidelines were published in the *Federal Register* on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for the hazardous substance described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a hazardous substance's toxicologic properties. Other pertinent literature is also presented, but is described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

The focus of the profiles is on health and toxicologic information; therefore, each toxicological profile begins with a public health statement that describes, in nontechnical language, a substance's relevant toxicological properties. Following the public health statement is information concerning levels of significant human exposure and, where known, significant health effects. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to protection of public health are identified by ATSDR and EPA.

Each profile includes the following:

(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a hazardous substance to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects;

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure that present a significant risk to human health of acute, subacute, and chronic health effects; and

(C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

The principal audiences for the toxicological profiles are health professionals at the Federal, State, and local levels; interested private sector organizations and groups; and members of the public.

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed. Staff of the Centers for Disease Control and Prevention and other Federal scientists have also reviewed the profile. In addition, this profile has been peer-reviewed by a nongovernmental panel and is being made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

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The toxicological profiles are developed in response to the Superfund Amendments and Reauthorization Act (SARA) of 1986 (Public Law 99-499) which amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). This public law directed ATSDR to prepare toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. The availability of the revised priority list of 275 hazardous substances was announced in the *Federal Register* on December 7, 2005 (70 FR 72840). For prior versions of the list of substances, see *Federal Register* notices dated April 17, 1987 (52 FR 12866); October 20, 1988 (53 FR 41280); October 26, 1989 (54 FR 43619); October 17, 1990 (55 FR 42067); October 17, 1991 (56 FR 52166); October 28, 1992 (57 FR 48801); February 28, 1994 (59 FR 9486); April 29, 1996 (61 FR 18744); November 17, 1997 (62 FR 61332); October 21, 1999 (64 FR 56792); October 25, 2001 (66 FR 54014); and November 7, 2003 (68 FR 63098). Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list.

QUICK REFERENCE FOR HEALTH CARE PROVIDERS

Toxicological Profiles are a unique compilation of toxicological information on a given hazardous substance. Each profile reflects a comprehensive and extensive evaluation, summary, and interpretation of available toxicologic and epidemiologic information on a substance. Health care providers treating patients potentially exposed to hazardous substances will find the following information helpful for fast answers to often-asked questions.

Primary Chapters/Sections of Interest

Chapter 1: Public Health Statement: The Public Health Statement can be a useful tool for educating patients about possible exposure to a hazardous substance. It explains a substance's relevant toxicologic properties in a nontechnical, question-and-answer format, and it includes a review of the general health effects observed following exposure.

Chapter 2: Relevance to Public Health: The Relevance to Public Health Section evaluates, interprets, and assesses the significance of toxicity data to human health.

Chapter 3: Health Effects: Specific health effects of a given hazardous compound are reported by type of health effect (death, systemic, immunologic, reproductive), by route of exposure, and by length of exposure (acute, intermediate, and chronic). In addition, both human and animal studies are reported in this section.
NOTE: Not all health effects reported in this section are necessarily observed in the clinical setting. Please refer to the Public Health Statement to identify general health effects observed following exposure.

Pediatrics: Four new sections have been added to each Toxicological Profile to address child health issues:

Section 1.6 How Can (Chemical X) Affect Children?
Section 1.7 How Can Families Reduce the Risk of Exposure to (Chemical X)?
Section 3.7 Children's Susceptibility
Section 6.6 Exposures of Children

Other Sections of Interest:

Section 3.8 Biomarkers of Exposure and Effect
Section 3.11 Methods for Reducing Toxic Effects

ATSDR Information Center

Phone: 1-800-CDC-INFO (800-232-4636) or 1-888-232-6348 (TTY) ***Fax:*** (770) 488-4178
E-mail: cdcinfo@cdc.gov ***Internet***: http://www.atsdr.cdc.gov

The following additional material can be ordered through the ATSDR Information Center:

Case Studies in Environmental Medicine: Taking an Exposure History—The importance of taking an exposure history and how to conduct one are described, and an example of a thorough exposure history is provided. Other case studies of interest include *Reproductive and Developmental Hazards; Skin Lesions and Environmental Exposures; Cholinesterase-Inhibiting Pesticide Toxicity*; and numerous chemical-specific case studies.

Managing Hazardous Materials Incidents is a three-volume set of recommendations for on-scene (prehospital) and hospital medical management of patients exposed during a hazardous materials incident. Volumes I and II are planning guides to assist first responders and hospital emergency department personnel in planning for incidents that involve hazardous materials. Volume III—*Medical Management Guidelines for Acute Chemical Exposure*s—is a guide for health care professionals treating patients exposed to hazardous materials.

Fact Sheets (ToxFAQs) provide answers to frequently asked questions about toxic substances.

Other Agencies and Organizations

The National Center for Environmental Health (NCEH) focuses on preventing or controlling disease, injury, and disability related to the interactions between people and their environment outside the workplace. Contact: NCEH, Mailstop F-29, 4770 Buford Highway, NE, Atlanta, GA 30341-3724 • Phone: 770-488-7000 • FAX: 770-488-7015.

The National Institute for Occupational Safety and Health (NIOSH) conducts research on occupational diseases and injuries, responds to requests for assistance by investigating problems of health and safety in the workplace, recommends standards to the Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA), and trains professionals in occupational safety and health. Contact: NIOSH, 200 Independence Avenue, SW, Washington, DC 20201 • Phone: 800-356-4674 or NIOSH Technical Information Branch, Robert A. Taft Laboratory, Mailstop C-19, 4676 Columbia Parkway, Cincinnati, OH 45226-1998 • Phone: 800-35-NIOSH.

The National Institute of Environmental Health Sciences (NIEHS) is the principal federal agency for biomedical research on the effects of chemical, physical, and biologic environmental agents on human health and well-being. Contact: NIEHS, PO Box 12233, 104 T.W. Alexander Drive, Research Triangle Park, NC 27709 • Phone: 919-541-3212.

Referrals

The Association of Occupational and Environmental Clinics (AOEC) has developed a network of clinics in the United States to provide expertise in occupational and environmental issues. Contact: AOEC, 1010 Vermont Avenue, NW, #513, Washington, DC 20005 • Phone: 202-347-4976 • FAX: 202-347-4950 • e-mail: AOEC@AOEC.ORG • Web Page: http://www.aoec.org/.

The American College of Occupational and Environmental Medicine (ACOEM) is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. Contact: ACOEM, 25 Northwest Point Boulevard, Suite 700, Elk Grove Village, IL 60007-1030 • Phone: 847-818-1800 • FAX: 847-818-9266.

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THE PROFILE HAS UNDERGONE THE FOLLOWING ATSDR INTERNAL REVIEWS:

1. Health Effects Review. The Health Effects Review Committee examines the health effects chapter of each profile for consistency and accuracy in interpreting health effects and classifying end points.

2. Minimal Risk Level Review. The Minimal Risk Level Workgroup considers issues relevant to substance-specific Minimal Risk Levels (MRLs), reviews the health effects database of each profile, and makes recommendations for derivation of MRLs.

3. Data Needs Review. The Applied Toxicology Branch reviews data needs sections to assure consistency across profiles and adherence to instructions in the Guidance.

4. Green Border Review. Green Border review assures the consistency with ATSDR policy.

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PEER REVIEW

A peer review panel was assembled for benzene. The panel consisted of the following members:

1. Dr. Jeffrey Fisher, Professor and Department Head, Department of Environmental Health Science, University of Georgia, Athens, Georgia;

2. Dr. Tee Guidotti, Chair and Professor, Department of Occupational and Environmental Health, School of Public Health and Sciences, The George Washington University Medical Center, Washington, DC; and

3. Dr. Rogene Henderson, Senior Scientist, Lovelace Respiratory Research Institute, Albuquerque, New Mexico.

These experts collectively have knowledge of benzene's physical and chemical properties, toxicokinetics, key health end points, mechanisms of action, human and animal exposure, and quantification of risk to humans. All reviewers were selected in conformity with the conditions for peer review specified in Section 104(I)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

Scientists from the Agency for Toxic Substances and Disease Registry (ATSDR) have reviewed the peer reviewers' comments and determined which comments will be included in the profile. A listing of the peer reviewers' comments not incorporated in the profile, with a brief explanation of the rationale for their exclusion, exists as part of the administrative record for this compound.

The citation of the peer review panel should not be understood to imply its approval of the profile's final content. The responsibility for the content of this profile lies with the ATSDR.

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1. PUBLIC HEALTH STATEMENT

This public health statement tells you about benzene and the effects of exposure to it.

The Environmental Protection Agency (EPA) identifies the most serious hazardous waste sites in the nation. These sites are then placed on the National Priorities List (NPL) and are targeted for long-term federal clean-up activities. Benzene has been found in at least 1,000 of the 1,684 current or former NPL sites. Although the total number of NPL sites evaluated for this substance is not known, the possibility exists that the number of sites at which benzene is found may increase in the future as more sites are evaluated. This information is important because these sites may be sources of exposure and exposure to this substance may harm you.

When a substance is released either from a large area, such as an industrial plant, or from a container, such as a drum or bottle, it enters the environment. Such a release does not always lead to exposure. You can be exposed to a substance only when you come in contact with it. You may be exposed by breathing, eating, or drinking the substance, or by skin contact.

If you are exposed to benzene, many factors will determine whether you will be harmed. These factors include the dose (how much), the duration (how long), and how you come in contact with it. You must also consider any other chemicals you are exposed to and your age, sex, diet, family traits, lifestyle, and state of health.

1.1 WHAT IS BENZENE?

Benzene, also known as benzol, is a colorless liquid with a sweet odor. Benzene evaporates into air very quickly and dissolves slightly in water. Benzene is highly flammable. Most people can begin to smell benzene in air at approximately 60 parts of benzene per million parts of air (ppm) and recognize it as benzene at 100 ppm. Most people can begin to taste benzene in water at 0.5–4.5 ppm. One part per million is approximately equal to one drop in 40 gallons. Benzene is found in air, water, and soil. Benzene comes from both industrial and natural sources.

Industrial Sources and Uses. Benzene was first discovered and isolated from coal tar in the 1800s. Today, benzene is made mostly from petroleum. Because of its wide use, benzene ranks in the top 20 in production volume for chemicals produced in the United States. Various industries use benzene to make other chemicals, such as styrene (for Styrofoam® and other plastics), cumene (for various resins), and cyclohexane (for nylon and synthetic fibers). Benzene is also used in the manufacturing of some types of rubbers, lubricants, dyes, detergents, drugs, and pesticides.

Natural Sources. Natural sources of benzene, which include gas emissions from volcanoes and forest fires, also contribute to the presence of benzene in the environment. Benzene is also present in crude oil and gasoline and cigarette smoke. For more information on characteristics and uses of benzene, see Chapters 4 and 5.

1.2 WHAT HAPPENS TO BENZENE WHEN IT ENTERS THE ENVIRONMENT?

Benzene is commonly found in the environment. Industrial processes are the main sources of benzene in the environment. Benzene levels in the air can be elevated by emissions from burning coal and oil, benzene waste and storage operations, motor vehicle exhaust, and evaporation from gasoline service stations. Tobacco smoke is another source of benzene in air, particularly indoors. Industrial discharge, disposal of products containing benzene, and gasoline leaks from underground storage tanks release benzene into water and soil.

Benzene can pass into air from water and soil surfaces. Once in the air, benzene reacts with other chemicals and breaks down within a few days. Benzene in the air can also be deposited on the ground by rain or snow.

Benzene in water and soil breaks down more slowly. Benzene is slightly soluble in water and can pass through the soil into underground water. Benzene in the environment does not build up in plants or animals. For more information on what happens to benzene after it gets into the environment, see Chapters 5 and 6.

1.3 HOW MIGHT I BE EXPOSED TO BENZENE?

Everyone is exposed to a small amount of benzene every day. You are exposed to benzene in the outdoor environment, in the workplace, and in the home. Exposure of the general population to benzene mainly occurs through breathing air that contains benzene. The major sources of benzene exposure are tobacco smoke, automobile service stations, exhaust from motor vehicles, and industrial emissions. Vapors (or gases) from products that contain benzene, such as glues, paints, furniture wax, and detergents, can also be a source of exposure. Auto exhaust and industrial emissions account for about 20% of the total national exposure to benzene. About half of the exposure to benzene in the United States results from smoking tobacco or from exposure to tobacco smoke. The average smoker (32 cigarettes per day) takes in about 1.8 milligrams (mg) of benzene per day. This amount is about 10 times the average daily intake of benzene by nonsmokers.

Measured levels of benzene in outdoor air have ranged from 0.02 to 34 parts of benzene per billion parts of air (ppb) (1 ppb is 1,000 times less than 1 ppm). People living in cities or industrial areas are generally exposed to higher levels of benzene in air than those living in rural areas. Benzene levels in the home are usually higher than outdoor levels. People may be exposed to higher levels of benzene in air by living near hazardous waste sites, petroleum refining operations, petrochemical manufacturing sites, or gas stations.

For most people, the level of exposure to benzene through food, beverages, or drinking water is not as high as through air. Drinking water typically contains less than 0.1 ppb benzene. Benzene has been detected in some bottled water, liquor, and food. Leakage from underground gasoline storage tanks or from landfills and hazardous waste sites that contain benzene can result in benzene contamination of well water. People with benzene-contaminated tap water can be exposed from drinking the water or eating foods prepared with the water. In addition, exposure can result from breathing in benzene while showering, bathing, or cooking with contaminated water.

Individuals employed in industries that make or use benzene may be exposed to the highest levels of benzene. As many as 238,000 people may be occupationally exposed to benzene in the United States. These industries include benzene production (petrochemicals, petroleum refining, and coke and coal chemical manufacturing), rubber tire manufacturing, and storage or transport of benzene and petroleum products containing benzene. Other workers who may be exposed to benzene include coke oven workers in the steel industry, printers, rubber workers, shoe makers, laboratory technicians, firefighters, and gas station employees. For more information on how you might be exposed to benzene, see Chapter 6.

1.4 HOW CAN BENZENE ENTER AND LEAVE MY BODY?

Benzene can enter your body through your lungs, gastrointestinal tract, and across your skin. When you are exposed to high levels of benzene in air, about half of the benzene you breathe in passes through the lining of your lungs and enters your bloodstream. When you are exposed to benzene in food or drink, most of the benzene you take in by mouth passes through the lining of your gastrointestinal tract and enters your bloodstream. A small amount will enter your body by passing through your skin and into your bloodstream during skin contact with benzene or benzene-containing products. Once in the bloodstream, benzene travels throughout your body and can be temporarily stored in the bone marrow and fat. Benzene is converted to products, called metabolites, in the liver and bone marrow. Some of the harmful effects of benzene exposure are caused by these metabolites. Most of the metabolites of benzene leave the body in the urine within 48 hours after exposure. For more information on how benzene can enter and leave your body, see Chapter 3.

1.5 HOW CAN BENZENE AFFECT MY HEALTH?

Scientists use many tests to protect the public from harmful effects of toxic chemicals and to find ways for treating persons who have been harmed.

One way to learn whether a chemical will harm people is to determine how the body absorbs, uses, and releases the chemical. For some chemicals, animal testing may be necessary. Animal testing may also help identify health effects such as cancer or birth defects. Without laboratory

animals, scientists would lose a basic method for getting information needed to make wise decisions that protect public health. Scientists have the responsibility to treat research animals with care and compassion. Scientists must comply with strict animal care guidelines because laws today protect the welfare of research animals.

After exposure to benzene, several factors determine whether harmful health effects will occur, as well as the type and severity of such health effects. These factors include the amount of benzene to which you are exposed and the length of time of the exposure. Most information on effects of long-term exposure to benzene are from studies of workers employed in industries that make or use benzene. These workers were exposed to levels of benzene in air far greater than the levels normally encountered by the general population. Current levels of benzene in workplace air are much lower than in the past. Because of this reduction and the availability of protective equipment such as respirators, fewer workers have symptoms of benzene poisoning.

Brief exposure (5–10 minutes) to very high levels of benzene in air (10,000–20,000 ppm) can result in death. Lower levels (700–3,000 ppm) can cause drowsiness, dizziness, rapid heart rate, headaches, tremors, confusion, and unconsciousness. In most cases, people will stop feeling these effects when they are no longer exposed and begin to breathe fresh air.

Eating foods or drinking liquids containing high levels of benzene can cause vomiting, irritation of the stomach, dizziness, sleepiness, convulsions, rapid heart rate, coma, and death. The health effects that may result from eating foods or drinking liquids containing lower levels of benzene are not known. If you spill benzene on your skin, it may cause redness and sores. Benzene in your eyes may cause general irritation and damage to your cornea.

Benzene causes problems in the blood. People who breathe benzene for long periods may experience harmful effects in the tissues that form blood cells, especially the bone marrow. These effects can disrupt normal blood production and cause a decrease in important blood components. A decrease in red blood cells can lead to anemia. Reduction in other components in the blood can cause excessive bleeding. Blood production may return to normal after exposure to benzene stops. Excessive exposure to benzene can be harmful to the immune

system, increasing the chance for infection and perhaps lowering the body's defense against cancer.

Long-term exposure to benzene can cause cancer of the blood-forming organs. This condition is called leukemia. Exposure to benzene has been associated with development of a particular type of leukemia called acute myeloid leukemia (AML). The Department of Health and Human Services has determined that benzene is a known carcinogen (can cause cancer). Both the International Agency for Cancer Research and the EPA have determined that benzene is carcinogenic to humans.

Exposure to benzene may be harmful to the reproductive organs. Some women workers who breathed high levels of benzene for many months had irregular menstrual periods. When examined, these women showed a decrease in the size of their ovaries. However, exact exposure levels were unknown, and the studies of these women did not prove that benzene caused these effects. It is not known what effects exposure to benzene might have on the developing fetus in pregnant women or on fertility in men. Studies with pregnant animals show that breathing benzene has harmful effects on the developing fetus. These effects include low birth weight, delayed bone formation, and bone marrow damage.

We do not know what human health effects might occur after long-term exposure to food and water contaminated with benzene. In animals, exposure to food or water contaminated with benzene can damage the blood and the immune system and can cause cancer. See Chapters 2 and 3 for more information on the health effects resulting from benzene exposure.

1.6 HOW CAN BENZENE AFFECT CHILDREN?

This section discusses potential health effects in humans from exposures during the period from conception to maturity at 18 years of age.

Children can be affected by benzene exposure in the same ways as adults. Benzene can pass from the mother's blood to a fetus. It is not known if children are more susceptible to benzene poisoning than adults.

1.7 HOW CAN FAMILIES REDUCE THE RISK OF EXPOSURE TO BENZENE?

If your doctor finds that you have been exposed to substantial amounts of benzene, ask whether your children might also have been exposed. Your doctor might need to ask your state health department to investigate.

Gasoline and cigarette smoke are two main sources of human exposure to benzene. Benzene exposure can be reduced by limiting contact with these sources. People are exposed to benzene from both active and passive second-hand smoke. Average smokers take in about 10 times more benzene than nonsmokers each day. Families are encouraged not to smoke in their house, in enclosed environments, or near their children.

Benzene is a major component of gasoline and used in many manufacturing processes. Increased levels of benzene can be found at fueling stations, and in air emissions from manufacturing plants and hazardous waste sites. Living near gasoline fueling stations or hazardous waste sites may increase exposure to benzene. People are advised not to have their families play near fueling stations, manufacturing plants, or hazardous waste sites.

1.8 IS THERE A MEDICAL TEST TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO BENZENE?

Several tests can show whether you have been exposed to benzene. Some of these tests may be available at your doctor's office. All of these tests are limited in what they can tell you. The test for measuring benzene in your breath must be done shortly after exposure. This test is not very helpful for detecting very low levels of benzene in your body. Benzene can be measured in your blood. However, because benzene rapidly disappears in the blood, measurements may be useful only for recent exposures.

In the body, benzene is converted to products called metabolites. Certain metabolites of benzene, such as phenol, muconic acid, and *S*-phenylmercapturic acid can be measured in the urine. The amount of phenol in urine has been used to check for benzene exposure in workers. The test is useful only when you are exposed to benzene in air at levels of 10 ppm or greater. However, this test must also be done shortly after exposure, and it is not a reliable indicator of how much benzene you have been exposed to, because phenol is present in the urine from other sources (diet, environment). Measurements of muconic acid or *S*-phenylmercapturic acid in the urine are more sensitive and reliable indicators of benzene exposure. The measurement of benzene in blood or of metabolites in urine cannot be used for making predictions about whether you will experience any harmful health effects. Blood counts of all components of the blood and examination of bone marrow are used to determine benzene exposure and its health effects.

For people exposed to relatively high levels of benzene, complete blood analyses can be used to monitor possible changes related to exposure. However, blood analyses are not useful when exposure levels are low. For more information on tests for benzene exposure, see Chapters 3 and 7.

1.9 WHAT RECOMMENDATIONS HAS THE FEDERAL GOVERNMENT MADE TO PROTECT HUMAN HEALTH?

The federal government develops regulations and recommendations to protect public health. Regulations *can* be enforced by law. The EPA, the Occupational Safety and Health Administration (OSHA), and the Food and Drug Administration (FDA) are some federal agencies that develop regulations for toxic substances. Recommendations provide valuable guidelines to protect public health, but *cannot* be enforced by law. The Agency for Toxic Substances and Disease Registry (ATSDR) and the National Institute for Occupational Safety and Health (NIOSH) are two federal organizations that develop recommendations for toxic substances.

Regulations and recommendations can be expressed as “not-to-exceed” levels, that is, levels of a toxic substance in air, water, soil, or food that do not exceed a critical value that is usually based on levels that affect animals; they are then adjusted to levels that will help protect humans.

Sometimes these not-to-exceed levels differ among federal organizations because they used different exposure times (an 8-hour workday or a 24-hour day), different animal studies, or other factors.

Recommendations and regulations are also updated periodically as more information becomes available. For the most current information, check with the federal agency or organization that provides it. Some regulations and recommendations for benzene include the following:

EPA has set 5 ppb as the maximum permissible level of benzene in drinking water. EPA has set a goal of 0 ppb for benzene in drinking water and in water such as rivers and lakes because benzene can cause leukemia. EPA estimates that 10 ppb benzene in drinking water that is consumed regularly or exposure to 0.4 ppb in air over a lifetime could cause a risk of one additional cancer case for every 100,000 exposed persons. EPA recommends 200 ppb as the maximum permissible level of benzene in water for short-term exposures (10 days) for children.

EPA requires that the National Response Center be notified following a discharge or spill into the environment of 10 pounds or more of benzene.

OSHA regulates levels of benzene in the workplace. The maximum allowable amount of benzene in workroom air during an 8-hour workday, 40-hour workweek is 1 ppm. Because benzene can cause cancer, NIOSH recommends that all workers wear special breathing equipment when they are likely to be exposed to benzene at levels exceeding the recommended (8-hour) exposure limit of 0.1 ppm. For more information on federal regulations, see Chapter 8.

1.10 WHERE CAN I GET MORE INFORMATION?

If you have any more questions or concerns, please contact your community or state health or environmental quality department, or contact ATSDR at the address and phone number below.

ATSDR can also tell you the location of occupational and environmental health clinics. These clinics specialize in recognizing, evaluating, and treating illnesses that result from exposure to hazardous substances.

Toxicological profiles are also available on-line at www.atsdr.cdc.gov and on CD-ROM. You may request a copy of the ATSDR ToxProfiles™ CD-ROM by calling the toll-free information and technical assistance number at 1-800-CDCINFO (1-800-232-4636), by e-mail at cdcinfo@cdc.gov, or by writing to:

Agency for Toxic Substances and Disease Registry
Division of Toxicology and Environmental Medicine
1600 Clifton Road NE
Mailstop F-32
Atlanta, GA 30333
Fax: 1-770-488-4178

Organizations for-profit may request copies of final Toxicological Profiles from the following:

National Technical Information Service (NTIS)
5285 Port Royal Road
Springfield, VA 22161
Phone: 1-800-553-6847 or 1-703-605-6000
Web site: http://www.ntis.gov/

2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO BENZENE IN THE UNITED STATES

Benzene is widely distributed in the environment. The exposure scenario of most concern to the general public is low-level inhalation over long periods. This is because the general population is exposed to benzene mainly through inhalation of contaminated air, particularly in areas of heavy traffic and around gas stations, and through inhalation of tobacco smoke from both active and passive smoking. Smoking has been identified as the single most important source of benzene exposure for the estimated 40 million U.S. smokers. Smoking accounts for approximately half of the total benzene exposure of the general population. Individuals employed in industries that make or use benzene, or products containing benzene, are probably exposed to the highest concentrations of atmospheric benzene. In addition, benzene is a common combustion product, providing high inhalation exposure potential for firefighters. Of the general population, those residing around certain chemical manufacturing sites or living near waste sites containing benzene or near leaking gasoline tanks may be exposed to concentrations of benzene that are higher than background air concentrations. In private homes, benzene levels in the air have been shown to be higher in homes with attached garages, or where the inhabitants smoke inside the house.

Although low levels of benzene have been detected in certain foods, beverages, and tap water, these do not constitute major sources of exposure for most people. However, leakage from underground gasoline storage tanks and seepage from landfills and hazardous waste sites have resulted in significant benzene contamination of well water. People with contaminated tap water can be exposed from drinking the water or eating foods prepared with it. In addition, exposure can also occur via inhalation during showering, bathing, or cooking with contaminated tap water. Showering and bathing with benzene-contaminated water can also contribute significantly to dermal exposure.

The Benzene Subregistry Baseline Technical Report of the National Exposure Registry contains information on 1,143 persons who had documented exposure to benzene in their drinking water and were exposed for at least 30 days. No causal relationship has been proposed for health conditions identified in the base subregistry or continued follow-up of the population.

2.2 SUMMARY OF HEALTH EFFECTS

The carcinogenicity of benzene is well documented in exposed workers. Epidemiological studies and case reports provide clear evidence of a causal relationship between occupational exposure to benzene and benzene-containing solvents and the occurrence of acute myelogenous leukemia (AML). The epidemiological studies are generally limited by confounding chemical exposures and methodological problems, including inadequate or lack of exposure monitoring and low statistical power, but a consistent excess risk of leukemia across studies indicates that benzene is the causal factor.

In vivo and *in vitro* data from both humans and animals indicate that benzene and/or its metabolites are genotoxic. Chromosomal aberrations (hypo- and hyperdiploidy, deletions, breaks, and gaps) in peripheral lymphocytes and bone marrow cells are the predominant effects seen in humans.

Damage to both the humoral and cellular components of the immune system has been known to occur in humans following inhalation exposure. This is manifested by decreased levels of antibodies and decreased levels of leukocytes in workers. Animal data support these findings.

The most characteristic systemic effect resulting from intermediate and chronic benzene exposure is arrested development of blood cells. Early biomarkers of exposure to relatively low levels of benzene include depressed numbers of one or more of the circulating blood cell types. A common clinical finding in benzene hematotoxicity is cytopenia, which is a decrease in various cellular elements of the circulating blood manifested as anemia, leukopenia, or thrombocytopenia in humans and in animals. Benzene-associated cytopenias vary and may involve a reduction in one (unicellular cytopenias) to all three (pancytopenia) cellular elements of the blood.

Benzene also causes a life-threatening disorder called aplastic anemia in humans and animals. This disorder is characterized by reduction of all cellular elements in the peripheral blood and in bone marrow, leading to fibrosis, an irreversible replacement of bone marrow. Benzene has also been associated with acute non-lymphocytic leukemia in humans, and aplastic anemia may be an early indicator of developing acute non-lymphocytic leukemia in some cases.

Limited information is available on other systemic effects reported in humans and is associated with high–level benzene exposure. Respiratory effects have been noted after acute exposure of humans to benzene vapors. Cardiovascular effects, particularly ventricular fibrillation, have been suggested as the

cause of death in fatal exposures to benzene vapor. Gastrointestinal effects have been noted in humans after fatal inhalation exposure (congestive gastritis), and ingestion (toxic gastritis and pyloric stenosis), of benzene. Myelofibrosis (a form of aplastic anemia) was reported by a gasoline station attendant who had been exposed to benzene by inhalation, and probably also through dermal contact. Myalgia was also reported in steel plant workers exposed to benzene vapors. Reports of renal effects in humans after benzene exposure consist of kidney congestion after fatal inhalation exposure. Dermal and ocular effects including skin irritation and burns, and eye irritation have been reported after exposure to benzene vapors. Swelling and edema have been reported to occur in a human who swallowed benzene. Studies in animals show systemic effects after inhalation exposure, including cardiovascular effects. Oral administration of benzene to animals has yielded information concerning hepatic effects. A study conducted in rabbits lends support to the finding that benzene is irritating and damaging to the skin and also shows that it is irritating and damaging to the eyes following dermal or ocular application.

Neurological effects have been commonly reported in humans following high-level exposure to benzene. Fatal inhalation exposure has been associated with vascular congestion in the brain. Chronic inhalation exposure has been associated with distal neuropathy, difficulty in sleeping, and memory loss. Oral exposure results in symptoms similar to inhalation exposure. Studies in animals suggest that inhalation exposure to benzene results in depressed electrical activity in the brain, loss of involuntary reflexes and narcosis, decrease in hind-limb grip strength and tremors, and narcosis, among other symptoms. Oral exposure to benzene has not been shown to cause significant changes in behavior. No neurological effects have been reported after dermal exposure to liquid benzene in either humans or animals.

Acute inhalation and oral exposures of humans to high concentrations of benzene have caused death. These exposures are also associated with central nervous system depression. Chronic low-level exposures have been associated with peripheral nervous system effects. Abnormalities in motor conduction velocity were noted in four of six pancytopenic individuals occupationally exposed to adhesives containing benzene.

Evidence of an effect of benzene exposure on human reproduction is not sufficient to demonstrate a causal association. Some animal studies provide limited evidence that benzene affects reproductive organs following inhalation exposure. Results from studies of benzene administered orally to rats and mice indicate no adverse effect on male or female reproductive organs at 17 weeks, but at 2 years, endometrial polyps were observed in female rats, preputial gland lesions were observed in male mice, and ovarian lesions were observed in female mice. Results are conflicting or inconclusive as to whether

inhalation of benzene vapors reduces the number of live fetuses and/or the incidences of pregnancy. Other studies are negative for effects on reproductive competence.

Epidemiological studies implicating benzene as a developmental toxicant have many limitations, and thus, it is not possible to assess the effect of benzene on the human fetus. Results of inhalation studies conducted in animals are fairly consistent across species and demonstrate that, at levels >47 ppm, benzene is fetotoxic as evidenced by decreased fetal weight and/or minor skeletal variants. Benzene has also been shown to reduce pup body weight in mice. A persistent decrease in the number of erythroid precursors was found in mice exposed *in utero*. Benzene has not been shown to be teratogenic, but has been shown to be fetotoxic in animals at high concentrations that are maternally toxic.

Cancer. The strongest evidence for the leukemogenic potential of benzene comes from series of cohort mortality studies on workers exposed to benzene in Ohio (the Pliofilm study) and China (the NCI/CAPM study). The Pliofilm study investigated workers exposed to benzene in three rubber hydrochloride ('Pliofilm') manufacturing plants. Mortality from all leukemias was increased but declined after additional years of follow-up, suggesting that the excess risk diminished with time since exposure. Exposures in the most recent 10 years were most strongly associated with leukemia risk, and there was no significant relation between leukemia death and benzene exposures received more than 20 years previously. AML accounted for most of the increased leukemia, and the risk of AML increased with increasing cumulative exposure above 200 ppm-years.

The NCI/CAPM study, a collaboration between the National Cancer Institute and the Chinese Academy of Preventive Medicine, evaluated lymphohematopoietic malignancies and other hematologic disorders in 74,828 benzene-exposed workers employed in 672 factories in 12 cities in China. Findings included increased risks for all leukemias, acute nonlymphocytic leukemia (ANLL), and combined ANLL and precursor myelodysplastic syndromes. These risks were increased at average exposure levels of 10–24 ppm and cumulative exposure levels of 40–99 ppm-years, and tended to increase with increasing average and cumulative levels of exposure.

The results of the Pliofilm and NCI/CAPM studies are consistent with epidemiologic studies and case reports showing increased incidences of leukemia in shoe factory and rotogravure plant workers exposed to high benzene levels during its use as a solvent. No significant increases in leukemia or other lymphohematopoietic malignancies were found in chemical industry workers or petroleum industry workers exposed to lower levels of benzene.

Possible associations between occupational exposure to benzene and non-Hodgkin's lymphoma (NHL) and multiple myeloma have been suggested. The risk for mortality from NHL increased with increasing level and duration of benzene exposure the NCI/CAPM study. The significance of this finding is unclear because NHL mortality was not significantly elevated in the cohort overall, concerns regarding the adequacy of the data have been raised, and increases in NHL were not found in other cohort mortality studies or in case-control studies of benzene-exposed workers.

The risk of mortality from multiple myeloma was increased in one of the early assessments of the Pliofilm cohort. The implication of this finding is unclear because the risk declined to non-significant levels in subsequent follow-up studies, and was not supported by the findings of other cohort mortality studies. Additionally, population-based and hospital-based case-control studies indicate that benzene exposure is not likely to be causally related to the risk of multiple myeloma. A meta-analysis of case-control studies found no significant association between occupational exposure to benzene and benzene-containing products and risk of multiple myeloma from sources categorized as benzene and/or organic solvents, petroleum, or petroleum products.

Animal studies provide supporting evidence for the carcinogenicity of benzene. Benzene has been shown to be a multiple site carcinogen in rats and mice following inhalation and oral exposure. Tumors that were increased in rats that were exposed to 200 or 300 ppm benzene by inhalation for 4–7 hours/day, 5 days/week for up to 104 weeks included carcinomas of the Zymbal gland and oral cavity. Mice that were exposed to 100 or 300 ppm benzene for 6 hours/day, 5 days/week for 16 weeks and observed for 18 months or life developed a variety of tumors, including thymic lymphomas, myelogenous leukemias, and Zymbal gland, ovarian, and lung tumors.

In oral bioassays conducted by the National Toxicology Program, benzene was administered to rats and mice by gavage at dose levels of 25–200 mg/kg/day on 5 days/week for 103 weeks. Tumors that were induced in the rats included Zymbal gland carcinomas and squamous cell papillomas and carcinomas of the oral cavity and skin. In the mice, benzene caused tumors that included malignant lymphomas, Zymbal gland carcinomas, lung alveolar/bronchiolar adenomas and carcinomas, Harderian gland adenomas, preputial gland squamous cell carcinomas, and mammary gland carcinomas. Similar effects occurred in rats exposed to 50–500 mg/kg/day benzene by gavage on 4–5 days/week for up to 104 weeks and observed for life; induced tumors included carcinomas of the Zymbal gland, oral cavity, forestomach,

nasal cavity, and skin. Mice that were similarly exposed to 500 mg/kg/day for 52 or 78 weeks developed Zymbal gland carcinomas, mammary carcinomas, and lung adenomas.

Application of benzene to the skin of animals has not produced evidence of carcinogenicity, although most of the dermal studies were inadequate for cancer evaluation. Many dermal carcinogenicity studies of other chemicals used benzene as a vehicle and treated large numbers of control animals (mice) with benzene alone. None of these studies indicated that benzene induced skin tumors; however, all possible tumor sites usually were not examined.

EPA, IARC, and the Department of Health and Human Services have concluded that benzene is a human carcinogen. The Department of Health and Human Services determined that benzene is a known carcinogen based on human evidence showing a causal relationship between exposure to benzene and cancer. Two studies classified benzene in Group 1 (carcinogenic to humans) based on sufficient evidence in both humans and animals. EPA classified benzene in Category A (known human carcinogen) based on convincing evidence in humans supported by evidence from animal studies. Under EPA's most recent guidelines for carcinogen risk assessment, benzene is characterized as a known human carcinogen for all routes of exposure based on convincing human evidence as well as supporting evidence from animal studies. Based on human leukemia data, EPA derived a range of inhalation unit risk values of $2.2x10^{-6}$–$7.8x10^{-6}$ $(\mu g/m^3)^{-1}$ for benzene. For risks ranging from $1x10^{-4}$ to $1x10^{-7}$, the corresponding air concentrations range from 13.0–45.0 $\mu g/m^3$ (4–14 ppb) to 0.013–0.045 $\mu g/m^3$ (0.004–0.014 ppb), respectively.

The consensus conclusion that benzene is a human carcinogen is based on sufficient inhalation data in humans supported by animal evidence, including the oral studies in animals. The human cancer induced by inhalation exposure to benzene is predominantly acute nonlymphocytic (myelocytic) leukemia, whereas benzene is a multiple site carcinogen in animals by both the inhalation and oral routes. Due to the lack of oral carcinogenicity data in humans, as well as the lack of a well-demonstrated and reproducible animal model for leukemia from benzene exposure, EPA extrapolated an oral slope factor from the inhalation unit risk range. The oral slope factor ranges from $1.5x10^{-2}$ to $5.5x10^{-2}$ $(mg/kg/day)^{-1}$, and for cancer risks from $1x10^{-4}$ to $1x10^{-7}$, the corresponding dose levels are $6.7x10^{-3}$–$1.8x10^{-3}$ to $6.7x10^{-6}$–$1.8x10^{-6}$ mg/kg/day, respectively.

Hematological Effects. Both human and animal studies have shown that benzene exerts toxic effects on various parts of the hematological system. All of the major types of blood cells are susceptible

(erythrocytes, leukocytes, and platelets). In the less severe cases of toxicity, specific deficiencies occur in individual types of blood elements. A more severe effect occurs when there is hypoplasia of the bone marrow, or hypercellular marrow exhibiting ineffective hematopoiesis so that all types of blood cells are found in reduced numbers. This is known as pancytopenia. A biphasic response (i.e., a hyperplastic effect in addition to destruction of the bone marrow cells) has been observed. Severe damage to the bone marrow involving cellular aplasia is known as aplastic anemia and can occur with prolonged exposure to benzene. This condition can lead to leukemia.

Numerous earlier studies of benzene-exposed workers demonstrated that chronic exposure to benzene air concentrations of 10 ppm or more resulted in adverse hematological effects, which increased in severity with increasing benzene exposure levels. Animal studies support the findings in humans. Significantly reduced counts for all three blood factors (white blood cells [WBCs], red blood cells [RBCs], and platelets); and other evidence of adverse effects on blood-forming units (reduced bone marrow cellularity, bone marrow hyperplasia and hypoplasia, granulocytic hyperplasia, decreased numbers of colony-forming granulopoietic stem cells and erythroid progenitor cells, damaged erythrocytes and erythroblast-forming cells) have been observed in animals at benzene concentrations in the range of 10–300 ppm and above.

Several more recent epidemiological studies have demonstrated hematological effects (including significant reductions in WBC, RBC, and platelet counts) in workers chronically exposed to benzene levels below 10 ppm, and even as low as 1 ppm or less. Results of one of these studies served as the basis for a chronic-duration inhalation MRL for benzene. Other reports demonstrated the lack of clinical signs of hematotoxicity following long-term, low-level occupational exposure to benzene levels below approximately 0.5 ppm (8-hour time-weighted average [TWA]). These investigators utilized a defined range of clinically normal hematological values and compared the prevalence of abnormal results between benzene-exposed workers and unexposed controls. The normal range for certain hematological parameters is necessarily broad due to large interindividual differences in clinical status. Restricting the comparison of benzene-exposed and nonexposed populations to only those values considered clinically abnormal or adverse may reduce the sensitivity of a particular study to detect meaningful changes at the population level.

Only one study was found that described hematological effects in humans after oral exposure to benzene. No reports describing hematological effects in humans following direct dermal exposure to benzene were found. However, intermediate- and chronic-duration animal studies show that loss of blood elements occurs in animals exposed to benzene in drinking water or by gavage at doses as low as 8–25 mg/kg/day.

Based on information found in the literature, it is reasonable to expect that adverse hematological effects might occur in humans after inhalation, oral, or dermal exposure, since absorption of benzene through any route of exposure would increase the risk of damage to blood elements. Studies show that the hematological system is susceptible to chronic exposure at low levels, so people living in and around hazardous waste sites that may be exposed to contaminated air, drinking water, soil, or food may be at an increased risk for adverse hematological effects. Deficiencies in various types of blood cells lead to other disorders, such as hemorrhagic conditions from a lack of platelets, susceptibility to infection from the lack of leukocytes, and increased cardiac output from the lack of erythrocytes.

Immunological and Lymphoreticular Effects. Benzene has been shown to have adverse immunological effects in humans following inhalation exposure for intermediate and chronic durations. Adverse immunological effects in animals occur following both inhalation and oral exposure for acute, intermediate, and chronic durations. The effects include damage to both humoral (antibody) and cellular (leukocyte) responses. Human studies of intermediate and chronic duration have shown that benzene causes decreases in the levels of circulating leukocytes in workers at low levels (30 ppm) of exposure and decreases in levels of circulating antibodies in workers exposed to benzene at 3–7 ppm. Other studies have shown decreases in human lymphocytes and other blood elements after exposure; these effects have been seen at occupational exposure levels as low as 1 ppm or less. Animal data support these findings. Both humans and rats have shown increases in leukocyte alkaline phosphatase activity. No studies regarding effects from oral or dermal exposure in humans were located. However, exposure to benzene through ingestion or dermal contact could cause immunological effects similar to those seen after inhalation exposure in humans and inhalation and oral exposure in animals.

Animal studies have also shown that benzene decreases circulating leukocytes and decreases the ability of lymphoid tissue to produce the mature lymphocytes necessary to form antibodies. This has been demonstrated in animals exposed for acute, intermediate, or chronic periods via the inhalation route. This decrease in lymphocyte numbers is reflected in impaired cell-mediated immune functions in mice following intermediate inhalation exposure to 100 ppm of benzene. The impaired cellular immunity after benzene treatment was observed both *in vivo* and *in vitro*. Mice exposed to 100 ppm for a total of 100 days were challenged with 10^4 polyoma virus-induced tumor cells (PYB6). Nine of 10 mice had reduced tumor resistance resulting in the development of lethal tumors. In the same study, lymphocytes were obtained from spleens of benzene-treated mice and tested for their immune capacity *in vitro*. The results showed that two other immune functions, alloantigen response (capacity to respond to foreign

antigens) and cytotoxicity, were also impaired. Similar effects were noted in mice exposed to benzene via the oral route for intermediate time periods, and in rats and mice exposed for chronic time periods. A decrease in spleen weight was observed in mice after acute-duration exposure to benzene at 25 ppm, the same dose levels at which a decrease in circulating leukocytes was observed. Similar effects on spleen weight and circulating leukocytes were observed in mice exposed to 12 ppm benzene 2 hours/day for 30 days. The acute-duration inhalation MRL was based on a study showing decreased mitogen-induced blastogenesis of B-lymphocytes following exposure of mice to benzene vapors at a concentration of 10 ppm, 6 hours/day for 6 days. The intermediate-duration inhalation MRL was based on a study showing delayed splenic lymphocyte reaction to foreign antigens evaluated by *in vitro* mixed lymphocyte culture following exposure of mice to benzene vapors at a concentration of 10 ppm, 6 hours/day, 5 days/week for a total of 20 exposures.

Based on information found in the literature, it is reasonable to expect that adverse immunological effects might occur in humans after inhalation, oral, or dermal exposure, since absorption of benzene through any route of exposure would increase the risk of damage to the immunological system. Studies show that the immunological system is susceptible to chronic exposure at low levels, so people living in and around hazardous waste sites who may be exposed to contaminated air, drinking water, soil, or food may be at an increased risk for adverse immunological effects.

Neurological Effects. In humans, results of occupational studies indicate that there is a cause-and-effect relationship between acute inhalation of very high concentrations of benzene and symptoms indicative of central nervous system toxicity. These symptoms, observed following both acute nonlethal and lethal exposures, include drowsiness, dizziness, headache, vertigo, tremor, delirium, and loss of consciousness. These symptoms are reversible when symptomatic workers are transferred from the problem area. Comparable toxicity in humans has been reported following ingestion of benzene at doses of 125 mg/kg and above. Occupational exposure to benzene has also been reported to produce neurological abnormalities in humans. Electromyographical and motor conduction velocity examinations were conducted on six patients with aplastic anemia, all of whom worked in environments where adhesives containing benzene were used (in one case, air concentrations bracketed around 210 ppm). Abnormalities in motor conduction velocity were noted in four of the six pancytopenic individuals and were thought to result from a direct effect of benzene on the peripheral nerves and/or spinal cord.

In its acute stages, benzene toxicity appears to be due primarily to the direct effects of benzene on the central nervous system, whereas the peripheral nervous system appears to be the target following chronic

low-level exposures. In addition, because benzene may induce an increase in brain catecholamines, it may also have a secondary effect on the immune system via the hypothalamus-pituitary-adrenal axis. Increased metabolism of catecholamines can result in increased adrenal corticosteroid levels, which are immunosuppressive.

Animal studies provide additional support that benzene affects the nervous system following acute inhalation and oral exposures, albeit at extremely high acute exposure levels. Effects reported include narcosis, nervous system depression, tremors, and convulsions. Acute and intermediate inhalation exposures have also been reported to produce adverse neurological effects in animals including a reduction in hind-limb grip strength and evoked electrical activity in the brain, and behavioral disturbances. Effects of benzene on learning were investigated in male hooded rats of the Sprague-Dawley strain given 550 mg/kg of benzene in corn oil or corn oil without benzene, intraperitoneally, on days 9, 11, and 13 postpartum. The rats exposed to benzene exhibited a significantly impaired learning ability when tested on problems of the closed-field, maze-learning task. This sign of neurotoxicity was not observed in control animals. In another study, 47-day-old juvenile cotton rats were maintained on one of two isocaloric diets containing either 4 or 16% crude protein for a 26-day experimental period. Animals were treated intraperitoneally with either 0 (corn oil), 100, 500, or 1,000 mg/kg benzene in corn oil for 3 consecutive days. The first dose was administered on days 15–17 of the experimental period. Animals were terminated on day 27. During the experimental period, severe loss of coordination was observed in some rats on the low protein diet immediately after exposure to benzene, but this subsided.

Intermediate oral exposures resulted in changes in the levels of monoamine transmitters in the brain without treatment-related behavioral changes. Mice exposed to 3 ppm for 2 hours/day for 30 days exhibited increased levels of acetylcholinesterase in the brain. *In vitro* studies suggest that benzene may have a direct effect on brain cells. Primary astrocyte cultures prepared from neonatal rat cerebella were treated with 3, 6, or 9 mmol/L benzene for 1 hour. ATPase and Mg^{2+}-ATPase activity were inhibited in a dose-related manner, and were detected at 78–92% of control values for ATPase, and 60–74% of control values for Mg^{2+}-ATPase.

These data suggest that humans exposed to benzene in the occupational setting for acute, intermediate, or chronic durations via the inhalation and oral routes are at risk of developing neurological effects. However, benzene levels in ambient air, drinking water, and at hazardous waste sites are lower and not likely to be of concern.

2.3 MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for benzene. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

Inhalation MRLs

- An MRL of 0.009 ppm has been derived for acute-duration inhalation exposure (14 days or less) to benzene.

The acute-duration inhalation MRL of 0.009 ppm was derived from a lowest-observed-adverse-effect level (LOAEL) value of 10.2 ppm for reduced lymphocyte proliferation following mitogen stimulation in mice (Rozen et al. 1984). The concentration was adjusted for intermittent exposure by multiplying the LOAEL (10.2 ppm) by 6 hours/24 hours to correct for less than a full day of exposure. The resulting adjusted LOAEL, 2.55 ppm, was then converted to a human equivalent concentration (HEC) according to EPA (1994b) methodology for calculating a HEC for extrarespiratory effects of a category 3 gas (such as benzene) as follows:

$$LOAEL_{HEC} = LOAEL_{ADJ} \times ([H_{b/g}]_A/[H_{b/g}]_H)$$

where:

$LOAEL_{HEC}$ = the LOAEL dosimetrically adjusted to a human equivalent concentration

$LOAEL_{ADJ}$ = the LOAEL adjusted from intermittent to continuous exposure

$[H_{b/g}]_A/[H_{b/g}]_H$ = the ratio of the blood:gas partition coefficient of the chemical for the laboratory animal species to the human value

If the animal blood:gas partition coefficient is greater than the human blood:gas partition coefficient, a default value of 1 is used for the ratio. According to Wiester et al. (2002), blood:gas partition coefficients for benzene in mice and humans are 17.44 and 8.12, respectively. Therefore, the default value of 1 is applied, in which case, the $LOAEL_{[HEC]}$ is equivalent to the $LOAEL_{[ADJ]}$.

Therefore:

$LOAEL_{HEC} = LOAEL_{ADJ} = 2.55$ ppm

The resulting $LOAEL_{(HEC)}$ of 2.55 ppm was then divided by an uncertainty factor of 300 (10 for the use of LOAEL, 3 for extrapolation from animals to humans using dosimetric conversion, and 10 for human variability) to yield the MRL value of 0.009 ppm (see Appendix A). An increased number of micronucleated polychromatic erythrocytes (MN-PCEs), decreased numbers of granulopoietic stem cells (Toft et al. 1982), lymphopenia (Cronkite et al. 1985), lymphocyte depression, and increased susceptibility to bacterial infection (Rosenthal and Snyder 1985) are among the adverse hematological and immunological effects observed in several other acute-duration inhalation studies. The study by Rozen et al. (1984) shows benzene immunotoxicity (reduced mitogen-induced lymphocyte proliferation) at a slightly lower exposure level than these other studies. C57BL/6J mice were exposed to 0, 10.2, 31, 100, and 301 ppm benzene for 6 hours/day for 6 days. Control mice were exposed to filtered, conditioned air only. Lymphocyte counts were depressed at all exposure levels; erythrocyte counts were elevated at 10.2 ppm, equal to controls at 31 ppm, and depressed at 100 and 301 ppm. Femoral B-lymphocyte and splenic B-lymphocyte numbers were reduced at 100 ppm. Levels of circulating lymphocytes and mitogen-induced blastogenesis of femoral B-lymphocytes were depressed after exposure to 10.2 ppm benzene for 6 days. Mitogen-induced blastogeneses of splenic T-lymphocytes were depressed after exposure to 31 ppm of benzene for 6 days. In another study, mice exhibited a 50% decrease in the

population of colony-forming granulopoietic stem cells (CFU-E) after exposure to 10 ppm benzene for 6 hours/day for 5 days (Dempster and Snyder 1991). In a study by Wells and Nerland (1991), groups of 4–5 male Swiss-Webster mice were exposed to 3, 25, 55, 105, 199, 303, 527, 1,150, or 2,290 ppm benzene for 6 hours/day for 5 days. The number of leukocytes in peripheral blood and spleen weights were significantly decreased compared with untreated controls at all concentrations >25 ppm. Therefore, 3 ppm was the no-observed-adverse-effect level (NOAEL) and 25 ppm was the LOAEL for these effects. These data support the choice of Rozen et al. (1984) as the study from which to derive the MRL.

- An MRL of 0.006 ppm has been derived for intermediate-duration inhalation exposure (15–364 days) to benzene.

The intermediate-duration inhalation MRL of 0.006 ppm was derived from a LOAEL value of 10 ppm for significantly delayed splenic lymphocyte reaction to foreign antigens evaluated in *in vitro* mixed lymphocyte reaction following the exposure of male C57Bl/6 mice to benzene vapors 6 hours/day, 5 days/week for 20 exposure days (Rosenthal and Snyder 1987). The concentration was adjusted for intermittent exposure by multiplying the LOAEL (10 ppm) by 6 hours/24 hours to correct for less than a full day of exposure and by 5 days/7days to correct for less than a full week of exposure. The resulting adjusted LOAEL, 1.8 ppm, was then converted to a HEC according to EPA (1994b) methodology for calculating a HEC for extrarespiratory effects of a category 3 gas (such as benzene) as follows:

$$LOAEL_{HEC} = LOAEL_{ADJ} \times ([H_{b/g}]_A/[H_{b/g}]_H)$$

where:

$LOAEL_{HEC}$ = the LOAEL dosimetrically adjusted to a human equivalent concentration

$LOAEL_{ADJ}$ = the LOAEL adjusted from intermittent to continuous exposure

$[H_{b/g}]_A/[H_{b/g}]_H$ = the ratio of the blood:gas partition coefficient of the chemical for the laboratory animal species to the human value

If the animal blood:gas partition coefficient is greater than the human blood:gas partition coefficient, a default value of 1 is used for the ratio. According to Wiester et al. (2002), blood:gas partition coefficients for benzene in mice and humans are 17.44 and 8.12, respectively. Therefore, the default value of 1 is applied, in which case, the $LOAEL_{[HEC]}$ is equivalent to the $LOAEL_{[ADJ]}$.

Therefore:

$LOAEL_{HEC} = LOAEL_{ADJ} = 1.8$ ppm

The resulting $LOAEL_{(HEC)}$ of 1.8 ppm was then divided by an uncertainty factor of 300 (10 for the use of LOAEL, 3 for extrapolation from animals to humans using dosimetric conversion, and 10 for human variability) to yield the MRL value of 0.006 ppm (see Appendix A).

Results of several studies support the choice of Rosenthal and Snyder (1987) as the basis for the intermediate-duration inhalation MRL for benzene (Baarson et al. 1984; Green et al. 1981a, 1981b), although the supporting studies employed a single exposure level, which precluded consideration as critical studies for MRL derivation. Exposure of C57BL mice to 10 ppm benzene for 6 hours/day, 5 days/week caused significant depressions in numbers of lymphocytes (ca. 30% lower than controls) as early as exposure day 32; this effect was also noted at the other scheduled periods of testing (exposure days 66 and 178) (Baarson et al. 1984). Splenic RBCs were significantly reduced (ca. 15% lower than controls) at exposure days 66 and 178. The failure of the erythrons of benzene-exposed mice to support normal red cell mass was illustrated by the significant reduction in peripheral red cell numbers in these animals at 66 and 178 days of benzene exposure. Green et al. (1981a, 1981b) exposed male CD-1 mice to benzene vapors at concentrations of 0 or 9.6 ppm for 6 hours/day, 5 days/week for 50 days and assessed the effects of exposure on cellularity in the spleen, bone marrow, and peripheral blood. Exposure-related effects included a 90% increase in numbers of multipotential hematopoietic stem cells (CFU-S) (Green et al. 1981a), approximately 25% increase in spleen weight and total splenic nucleated cellularity (Green et al. 1981b), and 80% increase in nucleated RBCs (Green et al. 1981b).

One intermediate-duration inhalation study reported increased rapid response to an electrical shock in mice at 0.78 ppm (Li et al. 1992). However, this study was not selected as the critical study for deriving an intermediate-duration inhalation MRL for benzene due to apparent discrepancies between reported and actual benzene exposure levels. Other animal studies identified neurological effects only at much higher exposure levels (Carpenter et al. 1944; Dempster et al. 1984; Evans et al. 1981; Frantik et al. 1994; Green et al. 1978).

- An MRL of 0.003 ppm has been derived for chronic-duration inhalation exposure (365 days or more) to benzene.

This MRL is based on statistically significantly decreased counts of B-lymphocytes in workers of shoe manufacturing industries in Tianjin, China (Lan et al. 2004a, 2004b), using a benchmark dose (BMD) analysis. The 250 benzene-exposed workers had been employed for an average of 6.1±2.9 years. Controls consisted of 140 age- and gender-matched workers in clothing manufacturing facilities in which measurable benzene concentrations were not found (detection limit 0.04 ppm). Benzene exposure was monitored by individual organic vapor monitors (full shift) 5 or more times during 16 months prior to phlebotomy. Benzene-exposed workers were categorized into four groups (140 controls, 109 at <1 ppm, 110 at 1–<10 ppm, and 31 at ≥10 ppm) according to mean benzene exposure levels measured during 1 month prior to phlebotomy. Complete blood count (CBC) and differential were analyzed mechanically. Coefficients of variation for all cell counts were <10%.

Mean 1-month benzene exposure levels in the four groups (controls, <1 ppm, 1–<10 ppm, and ≥10 ppm) were <0.04, 0.57±0.24, 2.85±2.11, and 28.73±20.74 ppm, respectively. Hematological values were adjusted to account for potential confounding factors (i.e., age, gender, cigarette smoking, alcohol consumption, recent infection, and body mass index). All types of WBCs and platelets were significantly decreased in the lowest exposure group (<1 ppm), ranging in magnitude from approximately 8 to 15% lower than controls. Although similar statistical analyses for the mid- and high-exposure groups were not included in the study report, decreases in all types of WBCs and platelets were noted at these exposure levels as well; the decreases in the highest exposure group ranged in magnitude from 15 to 36%. Lymphocyte subset analysis revealed significantly decreased CD4+-T cells, CD4+/CD8+ ratio, and B cells. Hemoglobin concentrations were significantly decreased only within the highest (≥10 ppm) exposure group. Tests for a linear trend using benzene air level as a continuous variable were significant for platelets and all WBC measures except monocytes and CD8+-T cells. Upon restricting the linear trend analyses to workers exposed to <10 ppm benzene, excluding controls, inverse associations remained for total WBCs, granulocytes, lymphocytes, B cells, and platelets. In order to evaluate the effect of past benzene exposures on the hematological effects observed in this study, the authors compared findings for a group of workers who had been exposed to <1 ppm benzene over the previous year (n=60) and a subset who also had <40 ppm-years lifetime cumulative benzene exposure (n=50). The authors stated that the same cell types were significantly reduced in these groups, but did not provide further information of the magnitude (i.e., percent change) of the hematological effects observed. These data suggest that the 1-month benzene exposure results could be used as an indicator of longer-term, low-level benzene hematotoxicity. To demonstrate that the observed effects were attributable to benzene, significantly decreased levels of WBCs, granulocytes, lymphocytes, and B cells were noted in a subgroup of the <1-ppm group for which exposure to other solvents was negligible.

As shown in Table A-1 of Appendix A, exposure-response relationships were noted for several blood factors. Benzene-induced decreased B cell count was selected as the critical effect for BMD modeling because it represented the highest magnitude of effect (i.e., B cell count in the highest exposure group was approximately 36% lower than that of controls). A BMD modeling approach was selected to identify the point of departure because the critical study (Lan et al. 2004a, 2004b) identified a LOAEL in the absence of a NOAEL.

As discussed in detail in Appendix A, the BMD analysis selected a point of departure ($BMCL_{0.25sd}$) of 0.1 ppm, which was adjusted from the 8-hour TWA to a continuous exposure concentration ($BMCL_{0.25sdADJ}$) using the default occupational minute volume (EPA 1994b). The resulting $BMCL_{0.25sdADJ}$ of 0.03 ppm was divided by an uncertainty factor of 10 (for human variability) to yield the chronic-duration inhalation MRL value of 0.003 ppm.

Several recent epidemiological studies provide supporting information to the Lan et al. (2004a, 2004b) findings of hematotoxicity in workers chronically exposed to relatively low levels of benzene (Qu et al. 2002, 2003a, 2003b; Rothman et al. 1996a, 1996b; Ward et al. 1996). Qu et al. (2002, 2003a, 2003b) compared hematologic values among 105 healthy workers (51 men, 54 women) in industries with a history of benzene usage (Tianjin, China) and 26 age-and gender-matched workers in industries that did not use benzene. A LOAEL of 2.26 ppm was identified for significantly reduced total WBCs, neutrophils, and RBCs; there was also an indication of benzene-induced changes in some hematological values at exposure levels lower than the current industry 8-hour TWA of 1 ppm. Rothman et al. (1996a, 1996b) identified an 8-hour TWA LOAEL of 7.6 ppm for a group of 11 benzene-exposed workers in a cross-sectional study of 44 healthy workers in Chinese (Shanghai) industries with a history of benzene usage and age- and gender-matched workers in industries that did not use benzene. In a nested case-control study of a cohort of workers in the Pliofilm production departments of a rubber products manufacturer in Ohio (Ward et al. 1996), a strong exposure-response relationship was correlated with low WBC count. A weak positive exposure-response relationship was observed for RBCs, which was significant for cumulative exposure up until the blood test date. The study authors noted that there was no evidence for a threshold for hematologic effects and suggested that exposure to benzene levels <5 ppm may result in hematologic suppression.

Oral MRLs

No acute-duration oral MRL was derived due to a lack of appropriate data on the effects of acute oral exposure to benzene.

In a study by Thienes and Haley (1972), central nervous system symptoms of giddiness, vertigo, muscular incoordination, unconsciousness, and death of humans have been reported when doses of ≥125 mg kg/body weight benzene were ingested. The only lower doses in the acute oral database are 50 mg/kg/day, at which pregnant rats experienced alopecia of hindlimbs and trunk (Exxon 1986), an end point not suitable for MRL derivation for benzene because the toxicological significance is not clear; and 88 mg/kg/day, a concentration resulting in slight non-dose-related central nervous system depression in rats (Cornish and Ryan 1965).

No intermediate-duration oral MRL was derived. Examination of the literature indicated that studies by Hsieh et al. (1988b, 1991) and Wolf et al. (1956) should be considered for derivation of an intermediate-duration oral MRL. Adult male CD-1 mice were exposed to 0, 8, 40, or 180 mg/kg/day benzene in the drinking water for 4 weeks (Hsieh et al. 1988b, 1991). Serious hematological and immunological effects (leukopenia, erythrocytopenia, lymphopenia, enhanced splenic lymphocyte proliferation) occurred at the lowest dose, precluding its use for MRL derivation. In the study by Wolf et al. (1956), a NOAEL of 1 mg/kg/day and a LOAEL of 50 mg/kg/day for leukopenia were identified in rats given benzene in oil by gavage for 6 months. However, closer examination of the study by Wolf et al. (1956) revealed that the results were not adequately supported by the data and analysis presented in the paper. Therefore, Wolf et al. (1956) was not selected for derivation of the MRL.

- An MRL of 0.0005 mg/kg/day has been derived for chronic-duration oral exposure (365 days or more) to benzene.

No human data are available to evaluate hematological effects following oral exposure to benzene. No adequate oral data were located from which a chronic-duration oral MRL for benzene could be derived, although a 2-year carcinogenesis bioassay of orally-exposed rats and mice is available (NTP 1986). Male rats were given 50, 100, or 200 mg/kg/day and female rats and mice of both sexes were given 0, 25, 50, and 100 mg/kg/day benzene in corn oil for 2 years. The dose of 25 mg/kg/day was a LOAEL for hematotoxicity and immunotoxicity in rats and mice, and is higher than the serious LOAEL of 8 mg/kg/day in the intermediate-duration database. Therefore, the threshold for hematological and immunological effects of benzene was not identified.

However, results of toxicokinetic studies of inhaled benzene in humans (Nomiyama and Nomiyama 1974a; Pekari et al. 1992; Srbova et al. 1950) and inhaled and orally-administered benzene in rats and mice (Sabourin et al. 1987) indicate that absorption of benzene at relatively low levels of exposure is approximately 50% of an inhaled dose and essentially 100% of an oral dose. Based on these assumptions, inhalation data can be used to estimate equivalent oral doses that would be expected to similarly affect the critical targets of benzene toxicity. Therefore, the point of departure for the chronic-duration inhalation MRL for benzene, namely the $BMCL_{0.25sdADJ}$ of 0.03 ppm for decreased B cell counts in benzene-exposed workers (Lan et al. 2004a, 2004b), serves as the point of departure for deriving the chronic-duration oral MRL as well.

The point of departure (in ppm) was converted to mg/m^3 using the molecular weight of 78.11 for benzene and assuming 25 °C and 760 mm Hg:

$$BMCL_{0.25sdADJ} \text{ of } 0.03 \text{ ppm} \times 78.11/24.45 = 0.096 \text{ mg/m}^3$$

The $BMCL_{0.25sdADJ}$ of 0.096 mg/m^3 for inhaled benzene was converted to an equivalent $BMDL_{0.25sdADJ}$ for ingested benzene using EPA (1988b) human reference values for inhalation rate (20 m^3/day) and body weight (70 kg) and a factor of 0.5 to adjust for differences in absorption of benzene following inhalation versus oral exposure (50 versus 100%, respectively) as follows:

$$BMDL_{0.25sdADJ} = BMCL_{0.25sdADJ} \text{ of } 0.096 \text{ mg/m}^3 \times 20 \text{ m}^3\text{/day} \times 0.5 \div 70 \text{ kg} = 0.014 \text{ mg/kg/day}$$

A total uncertainty factor of 30 (10 for human variability and 3 for uncertainty in route-to-route extrapolation) was applied to the $BMDL_{0.25sdADJ}$ of 0.014 mg/kg/day; the resulting chronic-duration oral MRL is 0.0005 mg/kg/day.

3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of benzene. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not

the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of benzene are indicated in Tables 3-1 and 3-2 and Figures 3-1 and 3-2. Because cancer effects could occur at lower exposure levels, Figures 3-1 and 3-2 also shows a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

3.2.1 Inhalation Exposure

Although occupational or environmental exposure to benzene or benzene-containing materials may include inhalation, oral, and dermal exposure routes, the inhalation and dermal routes are usually of primary concern in such scenarios. Data regarding occupational or environmental exposure in which inhalation is considered to have been the primary exposure route are summarized in this section (Section 3.2.1). Information regarding adverse health effects following oral or dermal exposure to benzene or benzene-containing materials is summarized in Sections 3.2.2 and 3.2.3, respectively.

3.2.1.1 Death

Case reports of fatalities due to acute benzene exposures have appeared in the literature since the early 1900s (Cronin 1924; Greenburg 1926; Hamilton 1922). Deaths occurred suddenly or within several hours after exposure (Avis and Hutton 1993; Cronin 1924; Greenburg 1926; Hamilton 1922; Winek et al. 1967). The benzene concentrations encountered by the victims were not often known. However, it has been estimated that 5–10 minutes of exposure to 20,000 ppm benzene in air is usually fatal (Flury 1928). Lethality in humans has been attributed to asphyxiation, respiratory arrest, central nervous system

depression, or suspected cardiac collapse (Avis and Hutton 1993; Hamilton 1922; Winek and Collom 1971; Winek et al. 1967). Cyanosis, hemolysis, and congestion or hemorrhage of organs were reported in the cases for which there were autopsy reports (Avis and Hutton 1993; Greenburg 1926; Hamilton 1922; Winek et al. 1967). No studies were located regarding noncancer-related mortality in humans following long-term inhalation exposure to benzene. Cancer-related mortality data for chronic-duration human occupational exposure to benzene are presented in Section 3.2.1.7.

In animals, acute inhalation exposure to high concentrations of benzene has caused death. An inhalation LC_{50} value for rats was calculated as 13,700 ppm for a 4-hour exposure (Drew and Fouts 1974). Additionally, 4 of 6 rats died following a 4-hour exposure to 16,000 ppm benzene (Smyth et al. 1962). However, in a study by Green et al. (1981b), male CD-1 mice exposed by inhalation to doses of benzene up to 4,862 ppm, 6 hours/day for 5 days showed no lethality. Lower doses (up to 400 ppm) for longer periods of time (2 weeks) did not cause death in mice (Cronkite et al. 1985). Lethality in monkeys and cats exposed to unspecified concentrations has been ascribed to ventricular fibrillation due to increased release of adrenaline (Nahum and Hoff 1934). Exposure of rabbits to 45,000 ppm of benzene for approximately 30 minutes caused narcosis that was followed by the death of all exposed animals (Carpenter et al. 1944). Furthermore, early deaths of rats and mice have occurred from intermediate and chronic exposure to air concentrations of 200 or 300 ppm of benzene in cancer studies (Cronkite et al. 1989; Farris et al. 1993; Maltoni et al. 1982a, 1983). Intermediate exposures (6 hours/day, 5 days/week for 50 days) of male CD-1 mice to benzene at doses of 9.6 ppm caused no increase in mortality, although mice exposed to 302 ppm benzene under the same regimen for a total of 26 weeks showed mortality approaching 50% (Green et al. 1981b). Mortality was observed in 97% of the CBA/Ca mice exposed to 300 ppm benzene for 16 weeks, as compared to 20% mortality in sham-exposed mice (Cronkite 1986). In Sprague-Dawley rats that received 300 ppm benzene vapor for 6 hours/day, 5 days/week for 691 days, the calculated median survival time was shown to be 51 weeks as compared to 65 weeks for controls (Snyder et al. 1978a). However, Snyder et al. (1984) reported a median survival time of 546 days for male Sprague-Dawley rats exposed to 100 ppm benzene for 5 days/week, 6 hours/day for life, compared to 560 days for air-exposed controls. It is not clear whether the difference in survival was significant or due to benzene exposure since both controls and exposed rats experienced early mortality from respiratory infections. Companion studies were also conducted with AKR and C57BL mice exposed to 300 ppm benzene (Snyder et al. 1978a, 1980). The calculated median survival time for AKR and C57BL mice exposed to 300 ppm benzene was shown to be 11 and 41 weeks compared to 39 and 75 weeks, respectively, for controls. For AKR mice exposed to 100 ppm, the calculated median survival time was 39 weeks (number of deaths not shown) as compared to 41 weeks for controls (Snyder et al. 1980).

The LC_{50} value and all reliable LOAEL values for each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.2 Systemic Effects

No studies were located regarding endocrine, metabolic, or body weight effects in humans or gastrointestinal, musculoskeletal, endocrine, metabolic, or dermal effects in animals following inhalation exposure to benzene. Available data pertaining to systemic effects are presented below.

The highest NOAEL values and all reliable LOAEL values for systemic effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

Respiratory Effects. Respiratory effects have been reported in humans after acute exposure to benzene vapors (Avis and Hutton 1993; Midzenski et al. 1992; Winek and Collom 1971; Winek et al. 1967; Yin et al. 1987b). Fifteen male workers employed in removing residual fuel from shipyard tanks were evaluated for benzene exposure (Midzenski et al. 1992). Mucous membrane irritation was noted in 80% and dyspnea was noted in 67% of the workers at occupational exposures of >60 ppm for up to 3 weeks. Nasal irritation and sore throat were reported by male and female workers exposed to 33 and 59 ppm benzene, respectively, for more than 1 year (Yin et al. 1987b). After a fatal occupational exposure to benzene vapors on a chemical cargo ship for only minutes, autopsy reports on three victims revealed hemorrhagic, edematous lungs (Avis and Hutton 1993). Acute granular tracheitis, laryngitis, bronchitis, and massive hemorrhages of the lungs were observed at autopsy of an 18-year-old male who died of benzene poisoning after intentional inhalation of benzene (Winek and Collom 1971). Similarly, acute pulmonary edema was found during the autopsy of a 16-year-old who died after sniffing glue containing benzene (Winek et al. 1967).

Snyder et al. (1978a, 1984) reported no treatment-related effects on lung tissue in male Sprague-Dawley rats exposed to 0, 100, or 300 ppm benzene 5 days/week, 6 hours/day for life. In addition, no adverse histopathological effects on lung tissue were observed in AKR/J or C57BL/65 mice exposed to 300 ppm benzene for life (Snyder et al. 1978a, 1980).

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Human	1 d 5-10 min				20000 (death)	Flury 1928	
2	Rat (Sprague-Dawley)	4 hr				13700 (LC50)	Drew and Fouts 1974	
3	Rat (NS)	4 hr				16000 (4/6 died)	Smyth et al. 1962	
4	Rabbit (NS)	3.7-36.2 min				45000 (death in 36.2 min)	Carpenter et al. 1944	
Systemic								
5	Human	1-21 d 2.5-8 hr/d	Resp		60 M (mucous membrane irritation, dyspnea)		Midzenski et al. 1992	
			Hemato			60 M (leukopenia, anemia, thrombocytopenia, MCV elevation)		
			Dermal		60 M (skin irritation)			
6	Rat (Sprague-Dawley)	Gd 6-15 6 hr/d	Bd Wt	300 F	2200 F (decreased maternal body weight)		Green et al. 1978	
7	Rat (Sprague-Dawley)	Gd 6-15 7 hr/d	Bd Wt	10 F	50 F (decreased maternal body weight and weight gain)		Kuna and Kapp 1981	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
8	Rat (Wistar)	7 d 8 hr/d	Hemato	50 F	100 F (leukopenia)		Li et al. 1986	
9	Rat (Wistar)	15 min	Cardio			3526 M (ventricular arrhythmia)	Magos et al. 1990	
10	Rat (CFY)	Gd 7-14 24 hr/d	Hepatic	125 F			Tatrai et al. 1980a	
			Bd Wt			125 F (decreased maternal weight gain of <22.08% of controls)		
11	Rat (CFY)	Gd 7-14 24 hr/d	Hepatic	47 F	141 F (increased relative liver weight)		Tatrai et al. 1980b	
			Bd Wt		47 F (decreased maternal weight gain)			
12	Rat (Sprague-Dawley)	2 wk 5 d/wk 6 hr/d	Hemato	30	300 (decrease in leukocytes, males; decrease in lymphocytes)		Ward et al. 1985	
13	Mouse (BALB/c)	7 d 6 hr/d	Hemato	47 M	211 M (depressed WBC count)		Aoyama 1986	
			Bd Wt	47 M	211 M (16% decrease in body weight)			

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
14	Mouse (BALB/c)	14 d 6 hr/d	Hemato		48 M (depressed WBC count)		Aoyama 1986	
			Bd Wt	48 M	208 M (18% decrease in body weight)			
15	Mouse (DBA/2)	2 wk 6 hr/d 5 d/wk	Hemato			300 M (hematocrit decreased by 26%, leukocytes decreased by 80%, bone marrow cellularity decreased by 93%)	Chertkov et al. 1992	
			Bd Wt		300 M (15% decrease)			
16	Mouse (Hale- Stoner)	11 d 5 d/wk 6 hr/d	Hemato			400 M (decreased erythrocytes and leukocytes)	Cronkite et al. 1982	
17	Mouse (C57B1/6 BNL)	2 wk 5 d/wk 6 hr/d	Hemato	25		100 (decreased hematocrit, hemolytic anemia)	Cronkite et al. 1985	
18	Mouse (Hale- Stoner)	2 d 5 d/wk 6 hr/d	Hemato		400 M (decreased CFU-E cells)		Cronkite et al. 1989	
19	Mouse (C57BL/6BNL)	8 d 6 hr/d	Hemato		3000 F (decreased marrow cellularity)		Cronkite et al. 1989	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
20	Mouse (DBA/2J)	5 d 6 hr/d	Hemato		10 M (50% decrease in CFU-E numbers)		Dempster and Snyder 1991	
21	Mouse (C57B1/6)	2-8 d 24 hr/d	Hemato			100 M (leukopenia; decrease in marrow cellularity)	Gill et al. 1980	
22	Mouse (CD-1)	5 d 6 hr/d	Hemato	9.9 M		103 M (decreased marrow cellularity; granulocytopenia, lymphocytopenia; decreased polymorphonucleucytes)	Green et al. 1981b	
			Bd Wt	4862 M				
23	Mouse (Swiss Webster, C57B1/6J)	2 wk 4 d/wk 6 h/d	Hemato			300 M (reduced bone marrow cellularity and CFU-E development)	Neun et al. 1992	
24	Mouse (Hybrid)	5 d 5 d/wk 6 hr/d	Hemato	300 F	900 F (CFU-E depression)		Plappert et al. 1994a	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
25	Mouse (C57BI/6J)	6 d 6 hr/d	Hemato		10.2 M (depressed lymphocyte counts; elevated RBCs)		Rozen et al. 1984	
26	Mouse (NMRI)	1-10 d 24 hr/d	Hemato			21 M (reduced bone marrow cellularity; increased polychromatic erythrocytes; decreased granulopoietic stem cells)	Toft et al. 1982	
27	Mouse (NMRI)	1 wk	Hemato	14 M			Toft et al. 1982	
28	Mouse (NMRI)	2 wk 5 d/wk 8 hr/d	Hemato	10.5 M	21 M (increased micronucleated polychromatic erythrocytes; decreased granulopoietic stem cells)		Toft et al. 1982	
29	Mouse (CD-1)	2 wk 5 d/wk 6 hr/d	Hemato	30		300 (anemia, decreased hemoglobin,erythrocytes and hematocrit; hypoplasia of bone marrow; leukopenia)	Ward et al. 1985	
			Bd Wt	300				

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
30	Mouse (Swiss-Webster)	5 d 6 hr/d	Hemato	3 M	25 M (decrease in WBC count)		Wells and Nerland 1991	
31	Rabbit	Gd 7-20 24 hr/d	Bd Wt	156.5 F	313 F (reduced maternal weight gain)		Ungvary and Tatrai 1985	
Immuno/ Lymphoret								
32	Rat (Wistar)	7 d 8 hr/d		50 F	100 F (leukopenia; increased leukocyte alkaline phosphatase)		Li et al. 1986	
33	Rat (Sprague-Dawley)	2 wk 5 d/wk 6 hr/d		200 M	400 M (29% reduction in total splenic cells, 28% lower thymus weight)		Robinson et al. 1997	
34	Mouse (BALB/c)	7 d 6 hr/d			47 M (depressed T- and B-lymphocytes; decreased spleen weight and WBC count)		Aoyama 1986	
35	Mouse (BALB/c)	14 d 6 hr/d			48 M (depressed T- and B-lymphocytes; decreased spleen and thymus weights and WBC count)		Aoyama 1986	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
36	Mouse (DBA/2)	2 wk 6 hr/d 5 d/wk				300 M (leukocytes decreased by 80%, bone marrow cellularity decreased by 93%)	Chertkov et al. 1992	
37	Mouse (CBA/Ca)	2 wk 5 d/wk 6 hr/d		10	25 (lymphopenia)		Cronkite 1986	
38	Mouse (Hale- Stoner)	11 d 5 d/wk 6 hr/d				400 M (decreased bone marrow cellularity)	Cronkite et al. 1982	
39	Mouse (C57B1/6 BNL)	2 wk 5 d/wk 6 hr/d		10	25 (lymphopenia)		Cronkite et al. 1985	
40	Mouse (CBA/Ca BNL)	2 d 5 d/wk 6 hr/d			3000 M (decreased lymphocytes, CFU-S content in marrow)		Cronkite et al. 1989	
41	Mouse (C57B1/6)	2-8 d 24 hr/d				100 M (leukopenia; decrease in marrow cellularity)	Gill et al. 1980	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
42	Mouse (CD-1)	5 d 6 hr/d		9.9 M		103 M (decreased femoral marrow and splenic cellularities; reduced splenic granulocytes)	Green et al. 1981a	
43	Mouse (Swiss Webster, C57B1/6J)	2 wk 4 d/wk 6 h/d				300 M (reduced bone marrow cellularity)	Neun et al. 1992	
44	Mouse (Hybrid)	5 d 5 d/wk 6 hr/d		100 F	300 F (increased helper lymphocytes)		Plappert et al. 1994a	
45	Mouse (C57BL/6)	1-12 d 6 hr/d		10 M	30 M (Listeria infection, T and B lymphocyte depression)		Rosenthal and Snyder 1985	
46	Mouse (C57BI/6J)	6 d 6 hr/d			10.2[b] M (decreased circulating lymphocytes and mitogen-induced blastogenesis of femoral T and B-lymphocytes)		Rozen et al. 1984	
47	Mouse (NMRI)	2 wk 5 d/wk 8 hr/d		10.5 M	21 M (decreased granulopoietic stem cells)		Toft et al. 1982	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
48	Mouse (NMRI)	1-10 d 24 hr/d			21 M (decreased granulopoietic stem cells)		Toft et al. 1982	
49	Mouse (CD-1)	2 wk 5 d/wk 6 hr/d		30		300 (leukopenia; lymphopenia; bone marrow hypoplasia; histopathological lesions in spleen, thymus, selected lymph nodes)	Ward et al. 1985	
50	Mouse (Swiss-Webster)	5 d 6 hr/d		3 M	25 M (decrease in spleen weight and WBC count)		Wells and Nerland 1991	
Neurological								
51	Human	30 min			300 (drowsiness, dizziness, headaches)		Flury 1928	
52	Human	1-21 d 2.5-8 hr/d			60 M (dizzyness, nausea, headache, peculiar or strong odor, chemical taste, fatigue)		Midzenski et al. 1992	
53	Rat (Sprague-Dawley)	Gd 6-15 6 hr/d		300 F	2200 F (lethargy)		Green et al. 1978	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
54	Mouse (C57BL)	1-14 d 5 d/wk 6 hr/d			100 M (increased milk licking: behavioral index)	3000 M (tremors; decreased grip strength)	Dempster et al. 1984	
55	Mouse (CD1, C57BL/6J)	5 d 6 hr/d			300 (hyperactivity)	900 (narcosis)	Evans et al. 1981	
56	Rabbit (NS)	3.7-36.2 min				45000 (narcosis, tremors, excitement, chewing, loss of pupillary and blink reflex; pupillary contraction and involuntary blinking)	Carpenter et al. 1944	
Reproductive								
57	Rat (Sprague-Dawley)	Gd 6-15 6 hr/d		100 F			Coate et al. 1984	
58	Rat (Sprague-Dawley)	Gd 6-15 6 hr/d		2200 F			Green et al. 1978	
59	Rat (CFY)	Gd 7-14 24 hr/d		125 F			Tatrai et al. 1980a	
60	Mouse (CF-1)	Gd 6-15 7 hr/d		500 F			Murray et al. 1979	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
61	Rabbit (New Zealand)	Gd 6-18 7 hr/d		500 F			Murray et al. 1979	
62	Rabbit	Gd 7-20 24 hr/d		156.5 F		313 F (increased abortions and resorptions)	Ungvary and Tatrai 1985	
Developmental								
63	Rat (Sprague-Dawley)	Gd 6-15 6 hr/d		40 F	100 F (decreased fetal weight)		Coate et al. 1984	
64	Rat (Sprague-Dawley)	Gd 6-15 6 hr/d			100 F (increased incidence of missing sternebrae)		Green et al. 1978	
65	Rat (Sprague-Dawley)	Gd 6-15 7 hr/d		10 F	50 F (decreased fetal weight)		Kuna and Kapp 1981	
66	Rat (CFY)	Gd 7-14 24 hr/d			125 F (decreased mean fetal weight; increased fetal weight retardation; skeletal retardation; 17% decrease in mean placental weight)		Tatrai et al. 1980a	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
67	Rat (CFY)	Gd 7-14 24 hr/d			47 F (decreased fetal weight; signs of skeletal retardation)	141 F (significant increase in fetal mortality)	Tatrai et al. 1980b	
68	Mouse (Swiss-Webster)	Gd 6-15 6 hr/d		10 F	20 F (decreased circulating erythroid precursors, elevation of granulocytic precourser cells in neonates and 6-week old offspring)		Keller and Snyder 1988	
69	Mouse (CF-1)	Gd 6-15 7 hr/d			500 F (decreased fetal body weight; delayed skeletal ossifications)		Murray et al. 1979	
70	Mouse (CFLP)	Gd 6-15 12 hr/d			156.5 F (decreased fetal body weight and skeletal retardation)		Ungvary and Tatrai 1985	
71	Rabbit (New Zealand)	Gd 6-18 7 hr/d			500 F (increased minor skeletal variants)		Murray et al. 1979	
72	Rabbit (New Zealand)	Gd 7-20 24 hr/d		156.5 F	313 F (decreased fetal weight; increased minor anomalies)		Ungvary and Tatrai 1985	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
INTERMEDIATE EXPOSURE								
Death								
73	Rat (Sprague-Dawley)	15 wk 4-5 d/wk 4-7 hr/d				200 (death)	Maltoni et al. 1983, 1985	
74	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d				300 M (97% mortality)	Cronkite 1986	
75	Mouse (CBA/Ca BNL)	16 wk 5 d/wk 6 hr/d				300 (deaths in males during exposure; deaths in females shortly after exposure)	Cronkite et al. 1989	
76	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d				300 M (11/125 died during first 9 months after initiation of exposure)	Farris et al. 1993	
77	Mouse (CD-1)	26 wk 5 d/wk 6 hr/d				302 M (50% mortality)	Green et al. 1981b	
Systemic								
78	Human	4 mo- 1 yr (occup)	Hemato			150 (pancytopenia)	Aksoy and Erdem 1978	
79	Human	4 mo -1 yr (occup)	Hemato			210 (pancytopenia, hypocellular to hypercellular bone marrow)	Aksoy et al. 1972	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
80	Human	1 yr (occup)	Hemato		40 (decrease in WBC counts in first 4 months)		Cody et al. 1993	
81	Human	3.5 mo- 19 yr (occup)	Hemato			29 (aplastic anemia)	Yin et al. 1987c	
82	Rat (Sprague-Dawley)	3 wk 5 d/wk 6 hr/d	Hemato			500 (decreased WBC and lymphocytes; increased RBC and hemoglobin)	Dow 1992	
83	Rat (Sprague-Dawley)	10 wk Gd 0-20 Ld 5-20 5 d/wk 6 hr/d	Bd Wt	300 F			Kuna et al. 1992	
84	Rat (Sprague-Dawley)	13 wk 5 d/wk 6 hr/d	Hemato	30		300 (decrease in leukocytes, slight decrease in marrow cellularity)	Ward et al. 1985	
			Bd Wt	300				
85	Rat (Wistar)	204 d 5 d/wk 7 hr/d	Hemato		88 M (leukopenia)		Wolf et al. 1956	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
86	Mouse (C57BL)	24 wk 5 d/wk 6 hr/d	Hemato		10 M (depressed peripheral red blood cells, CFU-E)		Baarson et al. 1984	The use of one dose precludes a dose-response assessment.
87	Mouse (C57BL)	24 wk 5 d/wk 6 hr/d	Hemato		10 M (depressed splenic red cells)		Baarson et al. 1984	The use of one dose precludes a dose-response assessment.
88	Mouse (Hale- Stoner)	9.5 wk 5 d/wk 6 hr/d	Hemato			400 M (decreased erythrocytes and leukocytes, decreased bone marrow cellularity)	Cronkite et al. 1982	
89	Mouse (C57B1/ 6BNL)	4-16 wk 5 d/wk 6 hr/d	Hemato		300 (stem cell depression in bone marrow, reversible after 2-4 weeks)		Cronkite et al. 1985	
90	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d	Hemato	25 M	316 M (decreased stem cells in bone marrow)		Cronkite et al. 1989	
91	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d	Hemato		300 M (granulocytic hyperplasia in bone marrow)		Farris et al. 1993	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
92	Mouse (B6C3F1)	up to 8 wk 5 d/wk 6 hr/d	Hemato	10 M	100 M (decreased numbers of differentiating and maturing hematopoietic bone marrow cells)		Farris et al. 1997a	
93	Mouse (CD-1)	26 wk 5 d/wk 6 hr/d	Hemato			302 M (decreased WBC, RBC; altered RBC morphology)	Green et al. 1981b	
			Bd Wt	302 M				
94	Mouse (CD-1)	50 d 5 d/wk 6 hr/d	Hemato	9.6 M			Green et al. 1981b	
			Bd Wt	9.6 M				
95	Mouse (Kunming)	30 d 6 d/wk 2 hr/d	Hepatic	12.52 M			Li et al. 1992	Exposures conducted under static conditions; measured only on first 3 of 30 days.
			Renal	12.52 M				
			Bd Wt	12.52 M				
96	Mouse (DBA/2, B6C3F1, C57B1/6)	13 wk 3 or 5 d/wk 6 hr/d	Hemato			300 M (depressed rate of erythropoiesis, increased frequency of MN-PCE and MN-NCE)	Luke et al. 1988b	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
97	Mouse (Hybrid)	8 wks 5 d/wk 6 hr/d	Hemato	100 F	300 F (slight anemia; BFU-E and CFU-E depression in bone marrow)		Plappert et al. 1994a	
98	Mouse (Hybrid)	8 wk 5 d/wk 6 hr/d	Hemato			300 F (decreased Hgb, Hct, erythrocyte counts)	Plappert et al. 1994b	
99	Mouse (BDF1)	8 wk 5 d/wk 6 hr/d	Hemato		100 F (BFU-E and CFU-E depression)		Seidel et al. 1989b	
100	Mouse (NMRI)	8 wk	Hemato	14 M			Toft et al. 1982	
101	Mouse (Hybrid)	6 or 7 wk 5 d/wk 6 h/d	Hemato			300 F (decreased CFU-C, BFU-E and CFU-E)	Vacha et al. 1990	
102	Mouse (CD-1)	13 wk 5 d/wk 6 hr/d	Hemato	30		300 (pancytopenia, bone marrow hypoplasia)	Ward et al. 1985	
			Bd Wt	300				
103	Gn Pig (NS)	32 or 269 d 5 d/wk 7 hr/d	Hemato		88 (leukopenia)		Wolf et al. 1956	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form	Comments
104	Rabbit (NS)	243 d 5 d/wk 7 hr/d	Hemato		80	(leukopenia)			Wolf et al. 1956	
105	Pig (Duroc-Jersey)	3 wk 5 d/wk 6 hr/d	Hemato	20	100	(decreased peripheral WBC, and increased erythroid cells)			Dow 1992	
Immuno/ Lymphoret										
106	Human	4 mo- 1 yr (occup)					210	(pancytopenia, hypoplastic to hyperplastic bone marrow, enlarged spleen)	Aksoy et al. 1972	
107	Human	1 yr (occup)			40	(decreased lymphocytes)			Cody et al. 1993	
108	Rat (Sprague-Dawley)	3 wk 5 d/wk 6 hr/d					500	(decreased myeloid and lymphoid cells)	Dow 1992	
109	Rat (Sprague-Dawley)	4 wk 5 d/wk 6 hr/d		200 M	400 M	(29% reduction in total splenic cells, 28% lower thymus weight)			Robinson et al. 1997	
110	Rat (Sprague-Dawley)	13 wk 5 d/wk 6 hr/d		30	300	(leukopenia and lymphopenia)			Ward et al. 1985	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
111	Rat (Wistar)	204 d 5 d/wk 7 hr/d			88 (leukopenia, increased spleen weight)		Wolf et al. 1956	
112	Rat (NS)	20 wk 6 d/wk 4 hr/d			4570 (increased leukocyte alkaline phosphatase, decreased white blood cell count)		Yin et al. 1982	
113	Mouse (C57B1)	24 wk 5 d/wk 6 hr/d			10 M (decreased number of splenic lymphocytes)		Baarson et al. 1984	The use of one dose precludes a dose-response assessment.
114	Mouse (C57B1/ 6BNL)	4-16 wk 5 d/wk 6 hr/d				300 (reduced bone marrow cellularity; stem cell depression, reversible after 2-4 weeks)	Cronkite et al. 1985	
115	Mouse (CBA/Ca BNL)	20 d 5 d/wk 6 hr/d				316 M (decreased lymphocytes, CFU-S content in marrow)	Cronkite et al. 1989	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
116	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d			300 M (granulocytic hyperplasia)		Farris et al. 1993	
117	Mouse (B6C3F1)	up to 8 wk 5 d/wk 6 hr/d		10 M	100 M (reduced numbers of total bone marrow cells, progenitor cells, differentiating hematopoietic cells, peripheral blood leukocytes and RBCs)		Farris et al. 1997a	
118	Mouse (B6C3F1)	8 wk 5 d/wk 6 hr/d		10 M	100 M (reduced lymphocyte and total nucleated cell counts)		Farris et al. 1997b	
119	Mouse (C57B1/6)	6 wk 5 d/wk 6 hr/d				1000 (leukopenia, granulocytopenia, lymphocytopenia)	Gill et al. 1980	
120	Mouse (CD-1)	50 d 6 hr/d 5 d/wk			9.6 M (increased splenic CFU-S)		Green et al. 1981a	The use of one exposure level precludes usefulness for dose-response assessment.

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
121	Mouse (CD-1)	26 wk 6 hr/d 5 d/wk				302 M (reduced marrow and spleen cellularity; decreased spleen weight)	Green et al. 1981a	
122	Mouse (CD-1)	50 d 5 d/wk 6 hr/d			9.6 M (increased spleen weight, total splenic nucleated cellularity and NRBC)		Green et al. 1981b	The use of one exposure level precludes usefulness for dose-response assessment.
123	Mouse (CD-1)	26 wk 5 d/wk 6 hr/d				302 M (lymphocytopenia, anemia, decreased spleen weight, decreased spleen and marrow cellularities)	Green et al. 1981b	
124	Mouse (Kunming)	30 d 6 d/wk 2 hr/d		3.13 M	12.52 M (26% decrease in relative spleen weight; decrease in myelocytes, premyelocytes, myeloblasts, and metamyelocytes in the bone marrow)		Li et al. 1992	Exposures conducted under static conditions; measured only on first 3 of 30 days.

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
125	Mouse (Hybrid)	8 wks 5 d/wk 6 hr/d		100 F	300 F (increased T4/T8 ratio)		Plappert et al. 1994a	
126	Mouse (C57B1/6)	20 d 5 d/wk 6 hr/d			10 M[c] (delayed splenic lymphocyte reaction to foreign antigens evaluated in in vitro mixed lymphocyte reaction)		Rosenthal and Snyder 1987	
127	Mouse (C57B1/6)	100 d 5 d/wk 6 hr/d				100 M (death in 9/10 mice due to depressed cell-mediated immunity)	Rosenthal and Snyder 1987	
128	Mouse (Hale- Stoner)	4-5 wk 5 d/wk 6 hr/d		50 F	200 F (suppressed antibody response to fluid tetanus toxoid)		Stoner et al. 1981	
129	Mouse (CD-1)	13 wk 5 d/wk 6 hr/d		30		300 (leukocyte & lymphocyte depression; bone marrow hypoplasia; histopathologic lesions in spleen and selected lymph nodes)	Ward et al. 1985	
130	Gn Pig (NS)	32 or 269 d 5 d/wk 7 hr/d			88 (leukopenia, increased spleen weight)		Wolf et al. 1956	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
131	Rabbit (NS)	243 d 5 d/wk 7 hr/d			80 (leukopenia)		Wolf et al. 1956	
132	Pig (Duroc-Jersey)	3 wk 5 d/wk 6 hr/d		20 F	100 F (T-cell depression; decreased peripheral WBC; decreased total lymphocytes)		Dow 1992	
Neurological								
133	Rat (Wistar)	3 wk 3-4 x 4 hr				929 M (calculated 30% depression of evoked electrical activity)	Frantik et al. 1994	
134	Mouse (H)	3 wk 3-4 x 2 hr				856 F (calculated 30% depression of evoked electrical activity)	Frantik et al. 1994	
135	Mouse (Kunming)	30 d 6 d/wk 2 hr/d			0.78 M (increased rapid response)		Li et al. 1992	Exposures conducted under static conditions; measured only on first 3 of 30 days.
Reproductive								
136	Rat (Sprague-Dawley)	10 wk Gd 0-20 Ld 5-20 5 d/wk 6 hr/d		300 F			Kuna et al. 1992	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
137	Rat (Wistar)	93 d 5 d/wk 7-8 hr/d			6600 M (testicular weight increase)		Wolf et al. 1956	
138	Mouse (CD-1)	13 wk 5 d/wk 6 hr/d		30		300 (bilateral cyst in ovaries; atrophy/degeneration of testes; decrease in spermatozoa; increase in abnormal sperm)	Ward et al. 1985	
139	Gn Pig (NS)	32 or 269 d 5 d/wk 7-8 hr/d			88 M (testicular weight increase)		Wolf et al. 1956	
140	Rabbit (NS)	243 d 5 d/wk 7-8 hr/d			80 M (degeneration of germinal epithelium in testes)		Wolf et al. 1956	
Cancer								
141	Human	3.5 mo- 19 yr (occup)				29 (CEL: humanlymphocytic leukemia)	Yin et al. 1987c	
142	Rat (Sprague-Dawley)	15 wk 4-5 d/wk 4-7 hr/d				200 (CEL: hepatomas)	Maltoni et al. 1982a, 1983, 1985	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
143	Rat (Sprague-Dawley)	15 wk 5 d/wk 4-7 hr/d				200 (CEL: hepatomas; onset of Zymbal gland carcinoma)	Maltoni et al. 1982b, 1983, 1985	
144	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d				100 M (CEL: leukemia)	Cronkite 1986	
145	Mouse (C57BL/ 6BNL)	4-16 wk 5 d/wk 6 hr/d				300 (CEL: thymic and non-thymic lymphoma)	Cronkite et al. 1984, 1985	
146	Mouse (CBA/Ca BNL)	16 wk 5 d/wk 6 hr/d				300 (Harderian and Zymbal gland, squamous cell and mammary carcinoma, papillary adenocarcinoma of the lung)	Cronkite et al. 1989	
147	Mouse (CBA/Ca BNL)	16 wk 5 d/wk 6 hr/d				100 M (CEL: hepatomata, lymphomatous and myelogenous neoplasms)	Cronkite et al. 1989	
148	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d				300 M (CEL: lymphoma in 12%)	Farris et al. 1993	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
149	Mouse (C57BL, CD-1)	10 wk 5 d/wk 6 hr/d				1200 M (CEL: 46% lung adenoma on CD-1 mice)	Snyder et al. 1988	
CHRONIC EXPOSURE								
Death								
150	Rat (Sprague-Dawley)	104 wk 5 d/wk 4-7 hr/d				200 (61% died, versus 46% in controls)	Maltoni et al. 1982a	
151	Rat (Sprague-Dawley)	691 d 5 d/wk 6 hr/d				300 M (median lifespan 51 weeks versus 65 weeks in controls)	Snyder et al. 1978a	
152	Mouse (AKR/J, C57Bl)	lifetime 5 d/wk 6 hr/d				300 M (median lifespan 11-41 weeks versus 39-75 weeks in controls)	Snyder et al. 1978a, 1980	
153	Mouse (CD-1)	222 d 5 d/wk 6 hr/d				300 M (decreased survival)	Snyder et al. 1982	
Systemic								
154	Human	4 mo- 15 yr (occup)	Hemato			150 (pancytopenia)	Aksoy and Erdem 1978	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
155	Human	14 yr (Occup)	Hemato	0.55			Collins et al. 1997	The NOAEL is for abnormal hematological values, not significantly altered values that would still fall within the range of normal values.
156	Human	1-3 yr (occup)	Hemato			3 (anemia, lymphocytosis, thrombocytopenia, leukopenia, leukocytosis)	Doskin 1971	
157	Human	NS (occup)	Hemato			24 (pancytopenia, hypoplastic bone marrow)	Erf and Rhoads 1939	
158	Human	3-29 yr (occup)	Hemato		25 M (increased mean corpuscular volume)		Fishbeck et al. 1978	
159	Human	0.5-5 yr (occup)	Hemato			11 (anemia, macrocytosis, thrombocytopenia)	Goldwater 1941, Greenburg et al. 1939	
160	Human	1-25 yr (occup)	Hemato	20		75 (anemia and leukopenia)	Kipen et al. 1989	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
161	Human	6.1 yr (avg) (Occup)	Hemato		0.57[d] (reduced WBC and platelet counts, approximately 7-18% lower than control values)		Lan et al. 2004a	
162	Human	4.5-9.7 yr (mean duration)	Hemato		2.26 (reduced neutrophils and RBC counts, approximately 12% lower than controls)		Qu et al. 2002, 2003	
163	Human	6.3 yr (avg) (Occup)	Hemato		7.6 (reduced absolute lymphocyte count, approximately 16% lower than controls)		Rothman et al. 1996a, 1996b	
164	Human	1-21 yr (occup)	Hemato	0.53 M			Tsai et al. 1983	
165	Human	>1 yr (occup)	Hemato		0.69 (leukopenia)		Xia et al. 1995	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
166	Human	>1 yr (occup)	Resp		33[e] M (sore throat; nasal irritation)		Yin et al. 1987b	
					59 F (sore throat; nasal irritation)			
			Hemato	33[e] M				
				59 F				
			Renal	33[e] M				
				59 F				
			Ocular		33[e] M (eye irritation)			
					59 F (eye irritation)			
167	Rat (Sprague-Dawley)	lifetime 5 d/wk 6 hr/d	Resp	300 M			Snyder et al. 1978a, 1984	
			Hemato			100 M (anemia, leukopenia)		
			Hepatic	300 M				
			Renal	300 M				
			Bd Wt	100 M	300 M (unspecified decreased body weight gain relative to controls)			

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
168	Mouse (AKR/J, C57BL/6J)	lifetime 5 d/wk 6 hr/d	Resp	300 M			Snyder et al. 1978a, 1980	
			Hemato			100 M (anemia, increased neutrophil levels; pancytopenia; bone marrow hypoplasia)		
			Hepatic	300 M				
			Renal	300 M				
			Bd Wt			300 M (59% decrease in weight gain)		
169	Mouse (CD-1)	222 d 5 d/wk 6 hr/d	Hemato		300 M (decreased RBCs and lymphocytes)		Snyder et al. 1982	
			Bd Wt		300 M (reduced body weight gain)			
Immuno/ Lymphoret								
170	Human	3-5 yr (occup)				11 (macrocytosis, thrombocytopenia)	Goldwater 1941, Greenburg et al. 1939	
171	Human	1-25 yr (occup)		20	75 (leukopenia)		Kipen et al. 1989	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
172	Human	>1 yr (occup)			0.69 (leukopenia)		Xia et al. 1995	
173	Rat (Sprague-Dawley)	lifetime 5 d/wk 6 hr/d			100 M (decreased lymphocyte counts, splenic hyperplasia)		Snyder et al. 1978a, 1984	
174	Mouse (AKR/J, C57Bl)	lifetime 5 d/wk 6 hr/d			100 M (lymphocytopenia, bone marrow hypoplasia)		Snyder et al. 1978a, 1980	
175	Mouse (C57BL, CD-1)	lifetime 6 hr/d 5 d/wk			300 M (lymphopenia)		Snyder et al. 1988	
Cancer								
176	Human	4-15 yr (occup)				150 (CEL: leukemia)	Aksoy and Erdem 1978	
177	Human	28 mo- 40 yr				1 (CEL: leukemia, lymphoma)	Aksoy et al. 1987	
178	Human	1-10 yr (occup) (occup)				10 M (CEL: leukemia)	Infante 1978; Infante 1977	
179	Human	18 mo (occup)				0.3 M (CEL: leukemia)	Ott et al. 1978	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
180	Human	1-14 yr (occup)				16 M (CEL: leukemia)	Rinsky et al. 1981; Infante et al. 1977a	
181	Human	1-30 yr (occup)				200 (CEL: leukemia)	Vigliani and Forni 1976	
182	Human	>1 yr (occup)				2 (CEL: chronic erythroid leukemia)	Yin et al. 1989	
183	Rat (Sprague-Dawley)	104 wk 5 d/wk 4-7 hr/d				200 (CEL: hepatomas)	Maltoni et al. 1982a	
184	Rat (Sprague-Dawley)	104 wk 5 d/wk 4-7 hr/d				200 (CEL: hepatomas)	Maltoni et al. 1983, 1985	
185	Rat (Sprague-Dawley)	lifetime 5 d/wk 6 hr/d				100 M (CEL: Zymbal gland carcinoma, myelogenous leukemia, liver tumors)	Snyder et al. 1984	
186	Mouse (AKR/J, C57BL6J)	lifetime 5 d/wk 6 hr/d				300 M (CEL: hematopoietic neoplasms [8/40], including 6 thymic lymphomas)	Snyder et al. 1978a, 1980	

Table 3-1 Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
187	Mouse (C57BL, CD-1)	lifetime every 3rd wk 7 d/wk				300 M (CEL: 35% increase of Zymbal gland carcinomas in C57BL mice)	Snyder et al. 1988	

a The number corresponds to entries in Figure 3-1.

b Used to derive an acute-duration inhalation minimal risk level (MRL) of 0.009 ppm for benzene. Concentration was adjusted for intermittent exposure by multiplying by 6 hours/24 hours and converted to a human equivalent concentration, which was divided by an uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans using dosimetric adjustment, and 10 for human variability) (see Appendix A).

c Used to derive an intermediate-duration inhalation minimal risk level (MRL) of 0.006 ppm for benzene. Concentration was adjusted for intermittent exposure by multiplying by 6 hours/24 hours and 5 days/7 days and converted to a human equivalent concentration, which was divided by an uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans using dosimetric adjustment, and 10 for human variability) (see Appendix A).

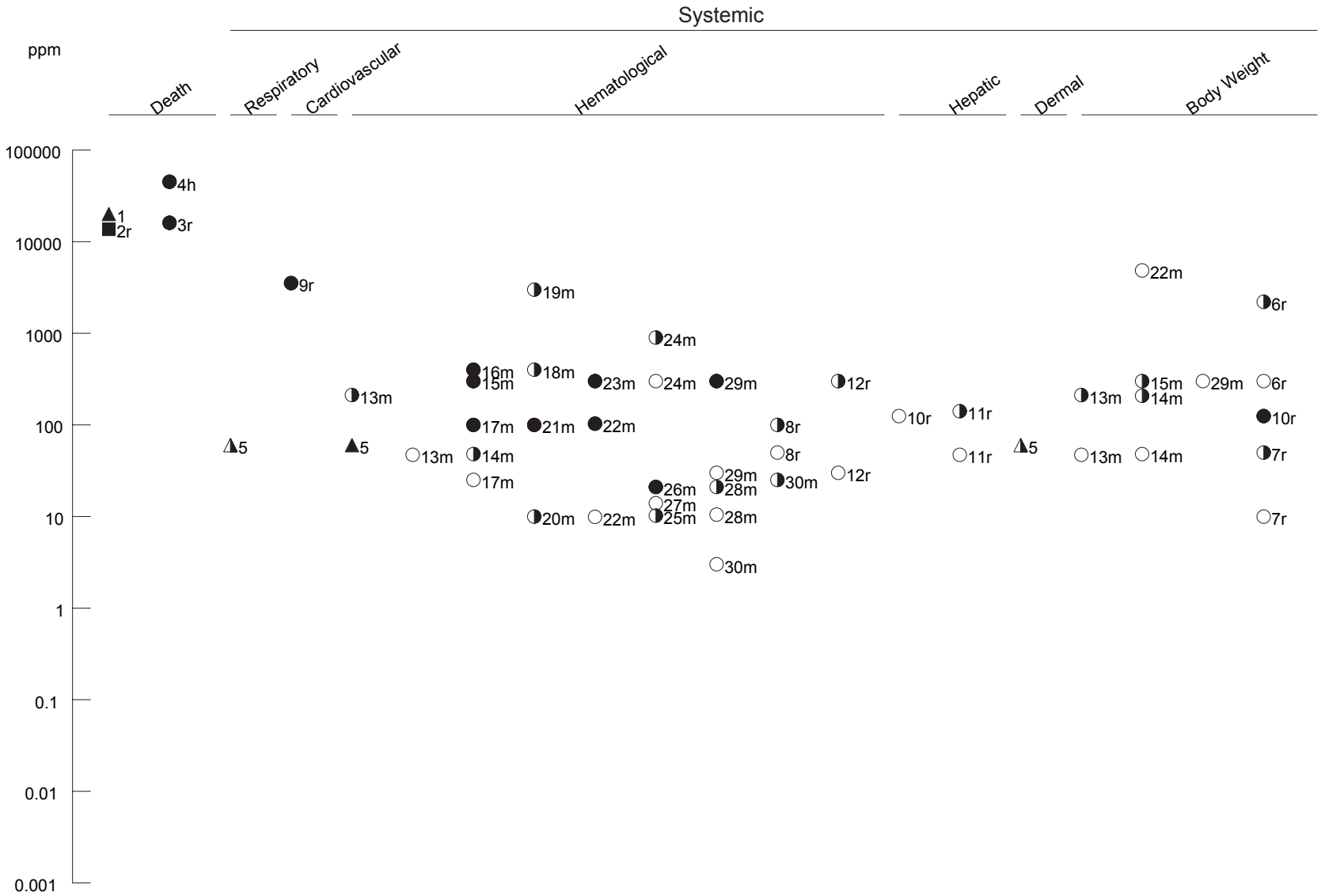
d Study results used to derive a chronic-duration inhalation minimal risk level (MRL) of 0.003 ppm for benzene, as described in detail in Appendix A. Benchmark dose (BMD) analysis was performed on B-lymphocyte counts to select a point of departure, which was adjusted for intermittent exposure and divided by an uncertainty factor of 10 for human variability. Study results also used to derive a chronic-duration oral minimal risk level (MRL) of 0.0005 mg/kg/day based on route-to-route extrapolation, as described in detail in Chapter 2 and Appendix A. Benchmark dose (BMD) analysis was performed on B-lymphocyte counts in benzene-exposed workers to select a point of departure, which was adjusted for intermittent exposure. An equivalent oral dose was estimated based on route-to-route extrapolation to determine a point of departure for deriving a chronic-duration oral MRL for benzene, which was divided by an uncertainty factor of 30 (10 for human variability and 3 for uncertainty in route-to-route extrapolation).

e Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 3-1. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

AChE = acetylcholinesterase; Bd Wt = body weight; BFU-E = burst-forming units - erythroid; Cardio = cardiovascular; CEL = cancer effect level; CFU-E = colony-forming units - erythroid progenitor cells; CFU-G = colony-forming units - granulopoietic stem cells; CFU-GM = colony-forming units - macrophages; CFU-S = colony-forming units - spleen; CNS = central nervous system; d = day(s); F = female; Gd = gestational day; Hct = hematocrit; Hemato = hematological; Hgb = hemoglobin; hr = hour(s); LC50 = lethal concentration, 50% kill; Ld = lactational day(s); LOAEL = lowest-observed-adverse-effect level; M = male; min = minute(s); MN-NCE = micronucleated normochromatic erythrocytes; MN-PCE = micronucleated polychromatic erythrocytes; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NRBC = nucleated red blood cells; NS = not specified; occup = occupational exposure; RBC = red blood cell; WBC = white blood cell; wk = week(s); yr = year(s)

Figure 3-1 Levels of Significant Exposure to Benzene - Inhalation

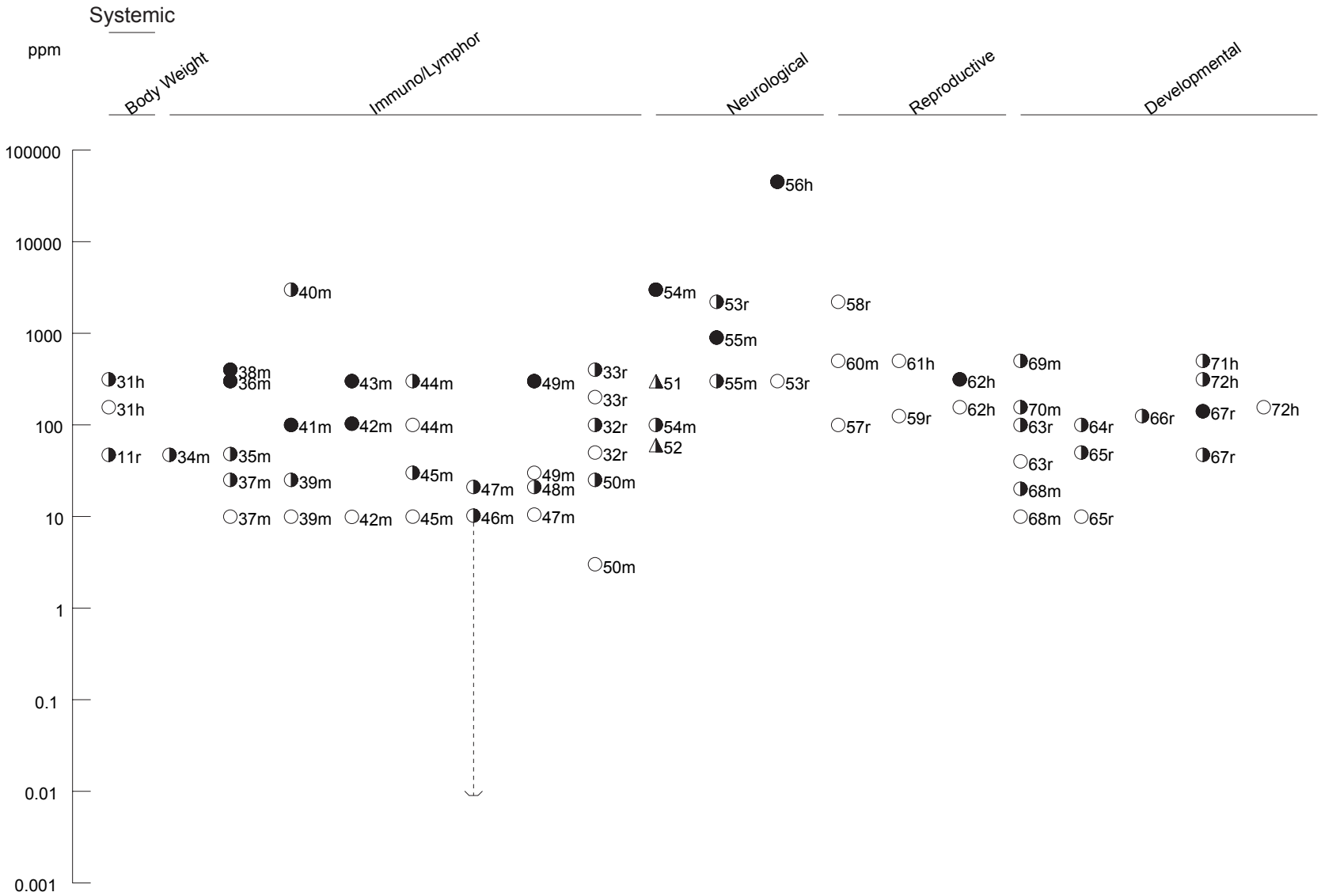
Acute (≤14 days)



c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level for effects other than Cancer
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◭ LOAEL, Less Serious-Humans	
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	
q-Cow	a-Sheep	g-Guinea Pig				

Figure 3-1 Levels of Significant Exposure to Benzene - Inhalation *(Continued)*

Acute (≤14 days)



c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level for effects other than Cancer
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◭ LOAEL, Less Serious-Humans	
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	
q-Cow	a-Sheep	g-Guinea Pig				

Figure 3-1 Levels of Significant Exposure to Benzene - Inhalation *(Continued)*

Intermediate (15-364 days)

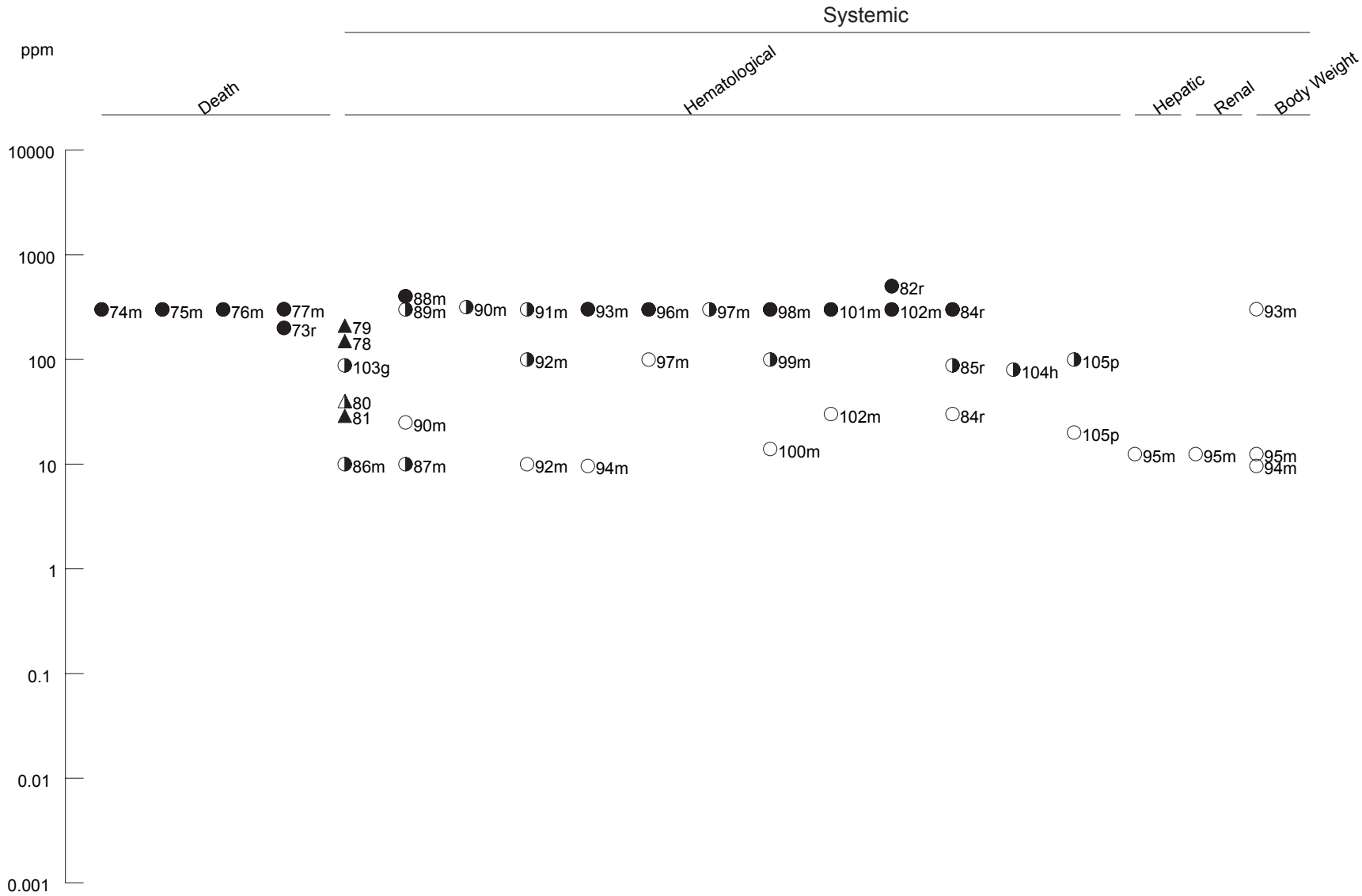
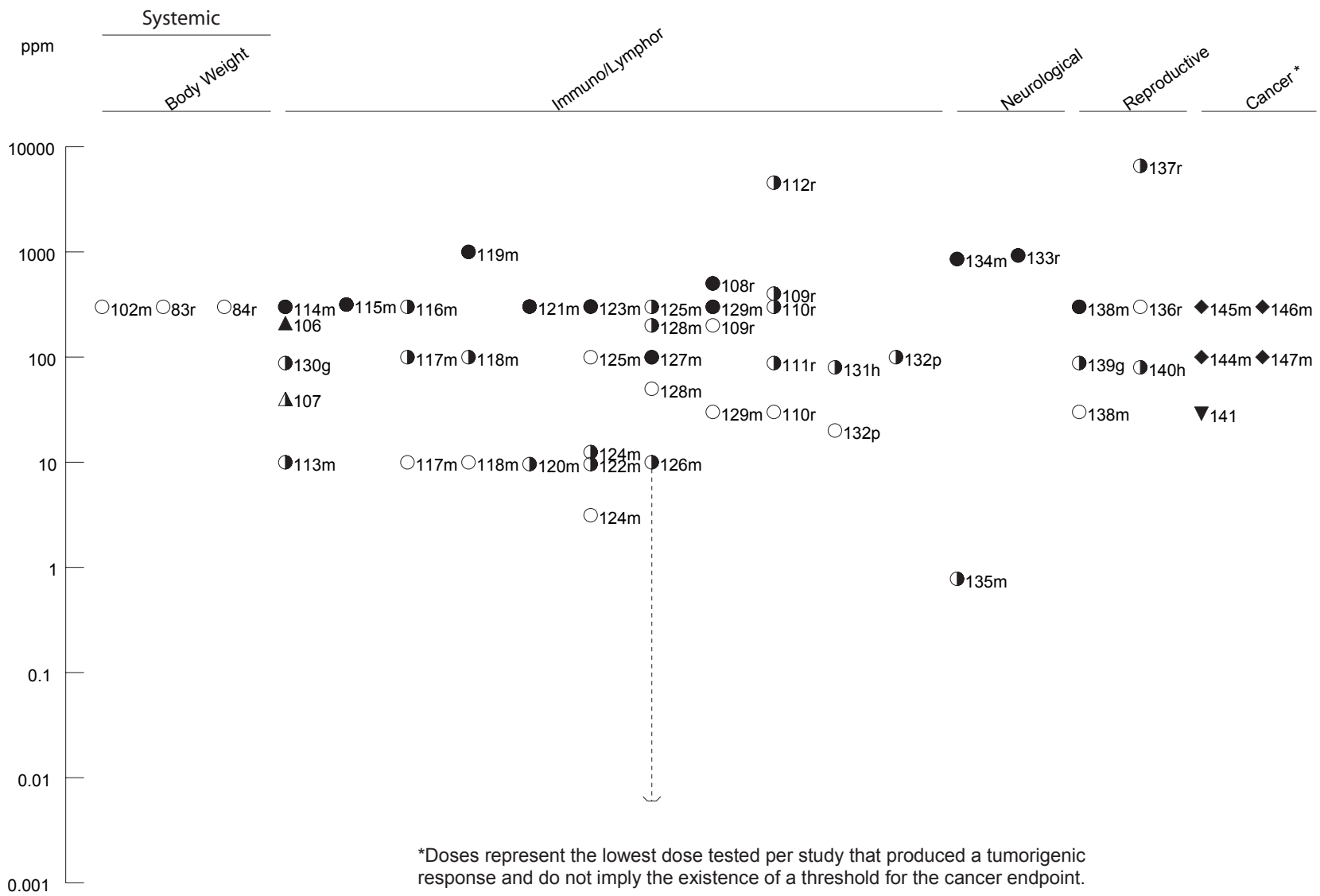


Figure 3-1 Levels of Significant Exposure to Benzene - Inhalation *(Continued)*

Intermediate (15-364 days)

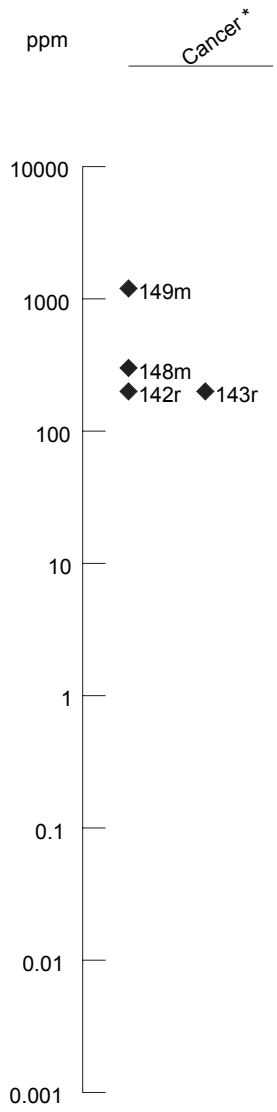


*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint.

c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level for effects other than Cancer
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◭ LOAEL, Less Serious-Humans	
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	
q-Cow	a-Sheep	g-Guinea Pig				

Figure 3-1 Levels of Significant Exposure to Benzene - Inhalation *(Continued)*

Intermediate (15-364 days)



*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint.

c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level for effects other than Cancer
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◭ LOAEL, Less Serious-Humans	
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	
q-Cow	a-Sheep	g-Guinea Pig				

Figure 3-1 Levels of Significant Exposure to Benzene - Inhalation *(Continued)*

Chronic (≥365 days)

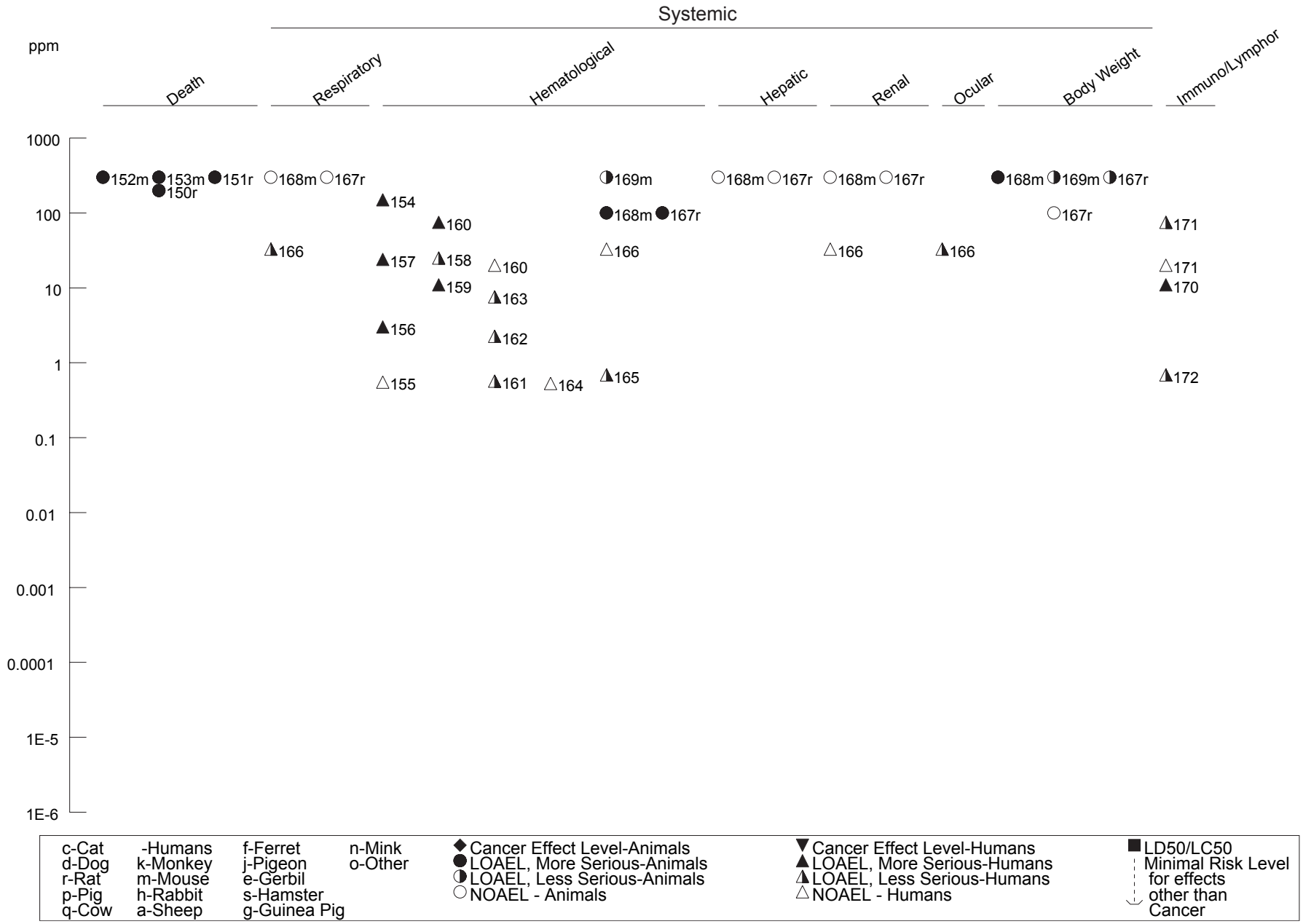
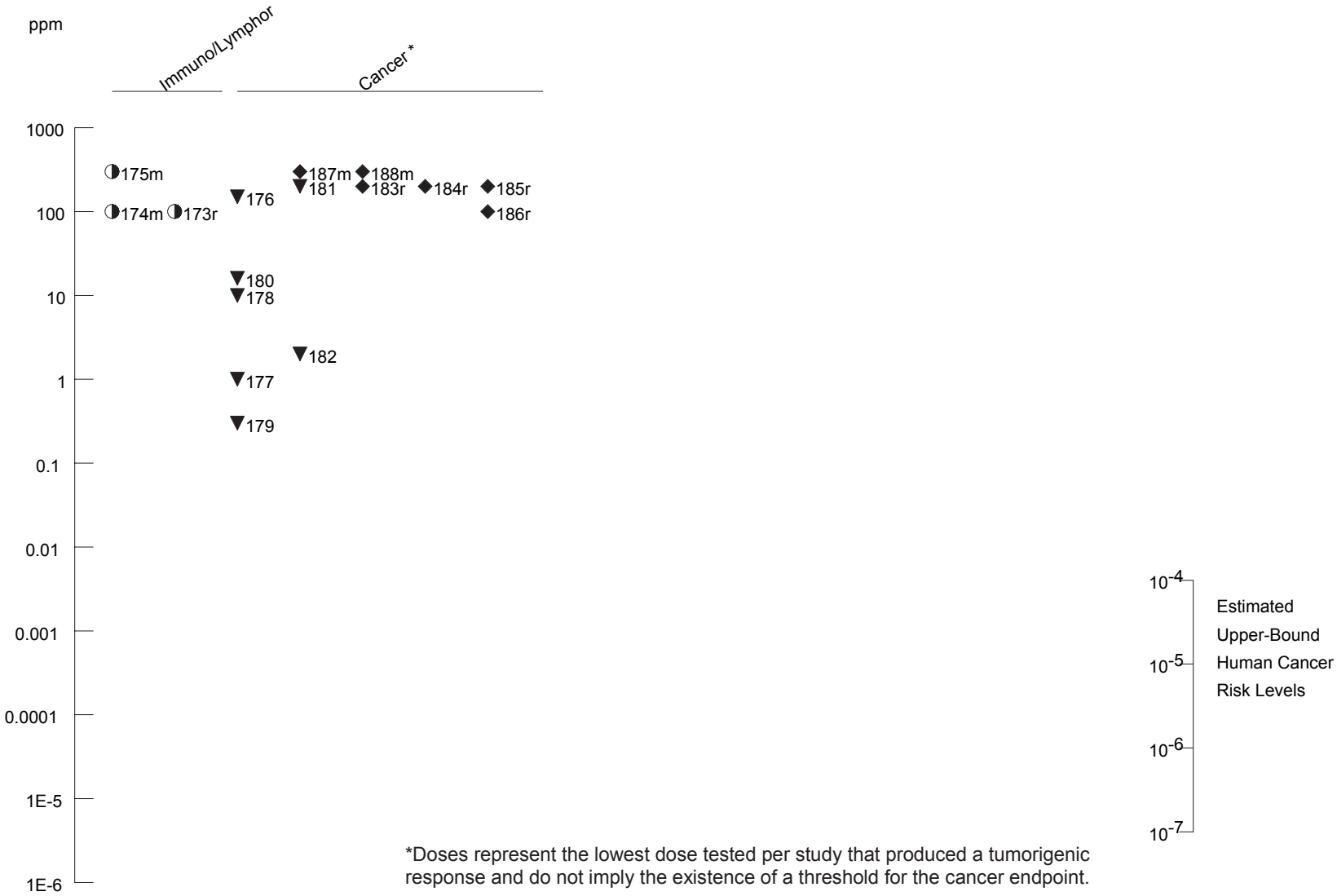


Figure 3-1 Levels of Significant Exposure to Benzene - Inhalation *(Continued)*

Chronic (≥365 days)



*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint.

c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level for effects other than Cancer
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◭ LOAEL, Less Serious-Humans	
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	
q-Cow	a-Sheep	g-Guinea Pig				

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after inhalation exposure to benzene, although ventricular fibrillation has been proposed as the cause of death in some human poisonings (Avis and Hutton 1993; Winek and Collom 1971).

One animal study was found that investigated the effects of acute inhalation exposure to high concentrations of benzene vapor on the heart muscle of cats and monkeys (Nahum and Hoff 1934). Information from the electrocardiograms indicated that exposure to benzene vapor caused extra systoles and ventricular tachycardia of the prefibrillation type. Animals that had their adrenals and stellate ganglias removed did not exhibit extra systoles or ventricular tachycardia. These findings suggest that the arrhythmias were caused by catecholamine release and sympathetic discharge. This study is limited in that exact levels of exposure are not available. An additional study investigated the influence of benzene inhalation on ventricular arrhythmia in the rat (Magos et al. 1990). Rats exposed to 3,526–8,224 ppm of benzene in a closed chamber for 15 minutes exhibited an increased number of ectopic ventricular beats.

Gastrointestinal Effects. Very few data are available describing gastrointestinal effects in humans after inhalation exposure to benzene. In a case study involving the death of an 18-year-old boy who intentionally inhaled benzene, the autopsy revealed congestive gastritis (Winek and Collom 1971). No other details or data were given.

Hematological Effects. Data regarding effects on the human hematological system following acute inhalation exposure to benzene are scant, but indicate leukopenia, anemia, and thrombocytopenia after more than 2 days of occupational exposure to more than 60 ppm benzene (Midzenski et al. 1992). Epidemiological studies on persons exposed to various levels of benzene in the workplace for intermediate and chronic periods of time also indicate hematological effects. Deficiencies in most of these studies include uncertainty in estimates of historical exposure levels, concomitant exposure to other chemicals, and lack of appropriate control groups. However, sufficient data are available to show that the hematopoietic system is a critical target for benzene toxicity. Studies that were conducted well and that show effects linked to specific exposure levels are presented in Table 3-1 and Figure 3-1. Effects on leukocytes, lymphocytes, and bone marrow are also discussed in Section 3.2.1.3.

Inhalation exposure to benzene levels in excess of regulated workplace limits (8-hour TWA of 1 ppm) for several months to several years can result in deficits in the relative numbers of circulating blood cells, which may be severe enough to be considered clinical pancytopenia. Continued exposure to benzene can

also result in aplastic anemia or leukemia (Aksoy et al. 1974; EPA 1995; Hayes et al. 1997; IARC 1982, 1987; IRIS 2007; Rinsky et al. 1987, 2002; Yin et al. 1987c, 1996a, 1996b).

Pancytopenia is the reduction in the number of all three major types of blood cells: erythrocytes (red blood cells), thrombocytes (platelets), and leukocytes (white blood cells). In adults, all three major types of blood cells are produced in the red bone marrow of the vertebrae, sternum, ribs, and pelvis. The red bone marrow contains immature cells, known as multipotent myeloid stem cells, that later differentiate into the various mature blood cells. Pancytopenia results from a reduction in the ability of the red bone marrow to produce adequate numbers of these mature blood cells.

Aplastic anemia is a more severe effect of benzene and occurs when the bone marrow ceases to function and the stem cells never reach maturity. Depression in bone marrow function occurs in two stages—hyperplasia (increased synthesis of blood cell elements), followed by hypoplasia (decreased synthesis). As the disease progresses, bone marrow function decreases and the bone marrow becomes necrotic and filled with fatty tissue. This myeloblastic dysplasia without acute leukemia has been seen in persons exposed to benzene (Erf and Rhoads 1939). Aplastic anemia can progress to a type of leukemia known as acute myelogenous leukemia (Aksoy 1980), which is discussed in Section 3.2.1.7.

Early biomarkers of exposure to relatively low levels of benzene include depressed numbers of one or more of the circulating blood cell types. For example, statistically significantly decreased total red blood cells (RBCs), white blood cells (WBCs), absolute lymphocyte count, platelets, and hematocrit were reported for a group of 44 healthy subjects exposed to benzene in the workplace (median 8-hour time-weighted average [TWA] of 31 ppm; minimal exposure to other solvents) for an average of 6.3 years in China (Rothman et al. 1996a, 1996b). Age- and gender-matched workers with no history of occupational exposure to benzene served as controls. Among the 22 workers whose mean 5-day benzene exposure levels did not exceed 31 ppm (median 8-hour TWA of 13.6 ppm), significantly depressed absolute lymphocyte count, RBCs, and platelets were noted. Only absolute lymphocyte count was significantly decreased in a subgroup of 11 workers with no 8-hour TWA exceeding 31 ppm (median 8-hour TWA of 7.6 ppm).

Qu et al. (2002, 2003a, 2003b) compared hematology values in a group of 130 chronically exposed workers in China with those obtained from 51 age- and gender-matched subjects without occupational exposure to benzene. Statistically significant trends for depressed RBCs, WBCs, and neutrophils were observed in the benzene-exposed workers (average measured 4-week benzene exposure levels ranged

from 0.08 to 54.5 ppm just prior to blood testing). A subgroup of 73 of these workers, whose average 4-week benzene exposure level was 2.26 ppm, exhibited significantly depressed RBCs and neutrophils. This study provided exposure-response data, but apparent discrepancies in reported low-concentration results render the study of limited value for MRL derivation.

One recent cross-sectional study (Lan et al. 2004a, 2004b), performed on 250 workers exposed to benzene in shoe manufacturing industries in Tianjin, China, and 140 age- and gender-matched workers in clothing manufacturing facilities that did not use benzene, was of sufficient quality to serve as the basis for deriving a chronic-duration inhalation MRL for benzene (see footnote to Table 3-1 and Appendix A). The benzene-exposed workers had been employed for an average of 6.1±2.9 years. Controls consisted of 140 age-and gender-matched workers in clothing manufacturing facilities in which measurable benzene concentrations were not found (detection limit 0.04 ppm). Benzene exposure was monitored by individual organic vapor monitors (full shift) 5 or more times during 16 months prior to phlebotomy. Benzene-exposed workers were categorized into four groups (controls, <1, 1–<10, and ≥10 ppm) according to mean benzene exposure levels measured during 1 month prior to phlebotomy. Complete blood count (CBC) and differential were analyzed mechanically. Coefficients of variation for all cell counts were <10%.

Mean 1-month benzene exposure levels in the four groups (controls, <1, 1–<10, and ≥10 ppm) were <0.04, 0.57±0.24, 2.85±2.11, and 28.73±20.74 ppm, respectively. Hematological values were adjusted to account for potential confounding factors (i.e., age, gender, cigarette smoking, alcohol consumption, recent infection, and body mass index). All types of WBCs and platelets were significantly decreased in the lowest exposure group (<1 ppm), ranging in magnitude from approximately 8 to 15% lower than controls. Although similar statistical analyses for the mid- and high-exposure groups were not included in the study report, decreases in all types of WBCs and platelets were noted at these exposure levels as well; the decreases in the highest exposure group ranged in magnitude from 15 to 36%. Lymphocyte subset analysis revealed significantly decreased CD4+-T cells, CD4+/CD8+ ratio, and B cells. Hemoglobin concentrations were significantly decreased only within the highest (≥10 ppm) exposure group. Tests for a linear trend using benzene air level as a continuous variable were significant for platelets and all WBC measures except monocytes and CD8+-T cells. Upon restricting the linear trend analyses to workers exposed to <10 ppm benzene, excluding controls, inverse associations remained for total WBCs, granulocytes, lymphocytes, B cells, and platelets. In order to evaluate the effect of past benzene exposures on the hematological effects observed in this study, the authors compared findings for a group of workers who had been exposed to <1 ppm benzene over the previous year (n=60) and a subset

who also had <40 ppm-years lifetime cumulative benzene exposure (n=50). The authors stated that the same cell types were significantly reduced in these groups, but did not provide further information of the magnitude (i.e., percent change) of the hematological effects observed. These data suggest that the 1-month benzene exposure results could be used as an indicator of longer-term low-level benzene hematotoxicity. To demonstrate that the observed effects were attributable to benzene, significantly decreased levels of WBCs, granulocytes, lymphocytes, and B cells were noted in a subgroup (n=30; mean 1-month exposure level of 0.29±0.15 ppm) of the <1 ppm group for which exposure to other solvents was negligible. Lan et al. (2004a, 2004b) also presented information on the effect of benzene on colony forming progenitor cells (data were only presented for the mid- and high-exposure groups). Benzene exposure was associated with a concentration-dependent decrease in colony formation and progenitor cells were suggested to be more sensitive than circulating cells.

As described above, several epidemiology studies compared hematological variables for benzene-exposed workers to gender- and age-matched controls and noted hematological effects at relatively low concentrations (well below 1 ppm in some exposure groups) (Lan et al. 2004a, 2004b; Qu et al. 2002, 2003a, 2003b; Rothman et al. 1996a, 1996b). An alternative approach was used by Collins et al. (1991, 1997) and Tsai et al. (1983, 2004). This approach utilized a defined range of clinically normal hematological values and compared the prevalence of abnormal results between benzene-exposed workers and unexposed controls. Collins et al. (1991) found no significant correlations between benzene exposure and the prevalence of abnormal hematological values among 200 workers exposed to benzene at estimated concentrations ranging from 0.01 to 1.4 ppm, relative to the prevalence of abnormal hematological values obtained from 268 unexposed workers in the same plant. In a more recent evaluation, Collins et al. (1997) found no significant correlation between exposure to benzene at an 8-hour TWA of 0.55 ppm and prevalence of clinically-defined lymphopenia (or other measures of hematotoxicity including mean corpuscular volume [MCV], and counts of WBCs, RBCs, hemoglobin, and platelets) among a group of 387 workers exposed for ≥5 years. Tsai et al. (1983) measured benzene levels in a Texas refinery at one time point (mean benzene concentration of 0.53 ppm) and found that hemoglobin, hematocrit, RBCs, WBCs, and thrombocytes of workers exposed for up to 21 years were within the range of normal values. Tsai et al. (2004) found no clinically adverse hematotoxic effects among a large group of 1,200 petrochemical employees with mean 8-hour TWA benzene exposure levels of 0.6 ppm from 1977 to 1988 and 0.14 ppm from 1988 to 2002. The normal range for certain hematological parameters is necessarily broad due to large interindividual differences in clinical status. Restricting the comparison of benzene-exposed and nonexposed populations to only those values

considered clinically abnormal or adverse may reduce the sensitivity of the study to detect meaningful changes at the population level.

Many reports of hematological effects in benzene-exposed workers involve estimated exposure levels well in excess of 1 ppm. A series of studies conducted on Turkish workers exposed to benzene-containing adhesives in various occupations showed increased severity of effects with increased levels or duration of exposure. The initial hematological study examined 217 male workers who were exposed for between 4 months and 17 years to benzene-containing solvents (Aksoy et al. 1971). The concentration of benzene in the work area ranged from 15 to 30 ppm outside work hours and reached a maximum of 210 ppm when benzene-containing adhesives were being used. Fifty-one of the workers showed clinical hematological abnormalities such as leukopenia, thrombocytopenia, eosinophilia, and pancytopenia. An additional cohort was identified that included 32 shoe manufacturers who had worked with benzene for 4 months to 15 years at concentrations of 15–30 ppm outside work hours and 210–640 ppm during the use of benzene, and who showed pancytopenia (Aksoy et al. 1972). Examination of these people revealed disruptions in bone marrow function, including cases of hypoplastic, acellular, hyperplastic, or normoblastic bone marrow. The continuing assessment further identified that ineffective erythropoiesis or increased hemolysis may have been responsible for the reticulocytosis, hyperbilirubinemia, erythroblastemia, increase in quantitative osmotic fragility, and elevated serum lactate dehydrogenase levels observed in some patients.

Leukopenia, lymphocytosis, a biphasic leukocyte response, and bone marrow hypercellularity were reported for workers in a Russian industry who were exposed to a complex of hydrocarbons that included benzene, cyclohexane, and 1,3-butadiene (Doskin 1971). Benzene levels were estimated at 3.2–12.8 ppm, which are 2–8 times the Russian standard. Leukopenia was observed by Xia et al. (1995) in Chinese workers exposed to 0.69–140 ppm (mean=6 ppm) benzene for more than 1 year. Enlarged RBCs, transient anemia, reduced hemoglobin concentrations, and clinically increased MCV were reported in workers in a chemical factory who were exposed to over 25 ppm of benzene in the workplace for an average of 9 years (Fishbeck et al. 1978).

Dosemeci et al. (1996) assessed the relative risk of abnormal hematological values with increasing benzene levels in a study of workers employed in 672 rubber and rubber glue application facilities in China between 1949 and 1987. Compared to workers with estimated exposures <5 ppm, relative risks of abnormal hematological values (indicating benzene poisoning) by estimated exposure intensity at 1.5 years prior to clinically-diagnosed benzene poisoning were 2.2 (95% confidence interval [CI] 1.7–

2.9), 4.7 (95% CI 3.4–6.5), and 7.2 (95% CI 5.3–9.8) for exposures in the ranges of 5–19, 20–39, and >40 ppm, respectively.

Examination of individual hematological records, demographic data, and chronological work histories of 459 workers employed at a rubber products manufacturing plant in Ohio between 1940 and 1975 revealed significant decreases in WBC and RBC counts and hemoglobin for the period of 1940–1948 when the exposure was 75 ppm (Kipen et al. 1989). The trend was not apparent during later years (1949–1975) when the exposure was decreased to 15–20 ppm. A more focused study of many of the same workers during their first year of employment revealed significantly lower WBC and RBC counts in employees exposed to benzene levels greater than the median (estimated at 40–54 ppm), compared to those with lower estimated exposure levels (Cody et al. 1993). Using a nested case-control approach at the same rubber products manufacturing plant to assess a possible exposure-response relationship between benzene and the risk of developing a low WBC or RBC count in workers for whom hematologic screening data were available, Ward et al. (1996) reported a strong correlation between low WBC counts and benzene exposure and a weak correlation between RBC count and benzene exposure.

More severe effects, including preleukemia or acute leukemia, were observed in 26 out of 28,500 benzene workers exposed to 210–650 ppm for 1–15 years (Aksoy et al. 1974). Clinical features of the preleukemia included one or more of the following: anemia, leukopenia, pancytopenia, bone marrow hyperplasia, pseudo-Pelger-Huet anomaly, and splenomegaly. A study was conducted 2–17 years following the last exposure of 44 pancytopenic patients exposed to benzene (150–650 ppm) in adhesives for 4 months to 15 years (Aksoy and Erdem 1978). Of these patients, complete remission was seen in 23, death due to complications of pancytopenia in 14, death due to myeloid metaplasia in 1, and leukemia in 6. When benzene concentrations in factories decreased in later years, less severe effects were seen. At 40 tire manufacturing plants, 231 workers were exposed for 28 months to 40 years (mean 8.8 years) to benzene-containing solvents and thinners (Aksoy et al. 1987). The decrease in benzene content of materials used in these workshops and the corresponding reduction in air concentration (most samples <1 ppm) paralleled the decrease in the number of hematological abnormalities reported for benzene-exposed workers.

Another study revealed effects, ranging from mild to severe, of benzene exposure in factory workers in China (Yin et al. 1987c). Of the 528,729 workers, 95% were exposed to mixtures of benzene, toluene, and xylene, while 5% (26,319 workers) were exposed to benzene alone at 0.02–264 ppm in air in 95% of the work stations. Over half of the work stations had levels of benzene in the air of less than 13 ppm;

about 1% had levels of 13–264 ppm. Benzene toxicity, as indicated by leukopenia, aplastic anemia, and leukemia, was seen in 0.94% of the workers exposed to benzene and 0.44% of the workers exposed to the mixtures. Similar toxicity was found in employees of 28 of the 141 shoe factories studied (124 cases in 2,740 employees) (Yin et al. 1987c). A positive correlation was observed for prevalence of adverse benzene effects and benzene concentration in data from these 28 shoe factories. The authors determined that the affected people were exposed to benzene concentrations >29 ppm. In one workshop, there were 4 cases of aplastic anemia in 211 workers. These workers were exposed to benzene at a mean concentration of 324 ppm during an 8-month period of employment. The prevalence of aplastic anemia in the shoe-making industry was about 5.8 times that in the general population. The main limitation of this study is the lack of information on the duration of exposure.

People who were exposed to high levels of benzene vapors in the printing industry also showed severe hematological effects. One study evaluated 332 workers who were exposed to 11–1,060 ppm of benzene for 6 months to 5 years. Detailed blood studies performed on 102 of these workers revealed benzene poisoning in 22 workers characterized by pancytopenia or other clinical signs (Goldwater 1941; Greenburg et al. 1939). Another study reported 6 cases of pancytopenia and bone marrow dysplasia in printers exposed to 24–1,060 ppm of benzene (Erf and Rhoads 1939).

Animal studies have been designed to characterize exposure-response relationships of benzene hematotoxicity; the results provide support to the human data. Animal responses to benzene exposure are variable and may depend on factors such as species, strain, duration of exposure, and whether exposure is intermittent or continuous. Wide variations have also been observed in normal hematological parameters, complicating statistical evaluation. However, the studies show that benzene exerts toxic effects at all phases of the hematological system, from stem cell depression in the bone marrow, to pancytopenia, to histopathological changes in the bone marrow. Effects on leukocytes, lymphocytes, and bone marrow are also discussed in Section 3.2.1.3.

Repeated acute-, intermediate-, and chronic-duration inhalation exposure of laboratory animals (mainly mice, but also rats, rabbits, and guinea pigs) to benzene vapor concentrations ranging from 10 to >300 ppm demonstrate significant decreases in blood values that include RBCs, total WBCs, lymphocytes, granulocytes, hematocrit, and hemoglobin (Aoyama 1986; Baarson et al. 1984; Chertkov et al. 1992; Cronkite et al. 1982; Farris et al. 1997a, 1997b; Gill et al. 1980; Green et al. 1981a, 1981b; Li et al. 1986; Rozen and Snyder 1985; Rozen et al. 1984; Snyder et al. 1982; Ward et al. 1985; Wells and Nerland 1991; Wolf et al. 1956). Pancytopenia was noted in the study of Ward et al. (1985).

Short-duration exposure of mice to benzene has been shown to cause both concentration- and duration-related reductions in bone marrow cellularity (number of nucleated cells) and the number of colony-forming granulopoietic stem cells (CFU-C), and an increased frequency of micronucleated polychromatic erythrocytes (MN-PCE) (Toft et al. 1982). Mice that were exposed continuously at benzene concentrations ≥21 ppm in air for 4–10 days showed significant changes in all three parameters. Intermittent exposure (8 hours/day, 5 days/week for 2 weeks) to 21 ppm significantly reduced the number of CFU-C and elevated the frequency of MN-PCE, but did not affect bone marrow cellularity. Intermittent exposure at concentrations ≥50 ppm caused significant changes in all three parameters and decreased the ability of the spleen to form mature cells (as measured by numbers of colony-forming units of stem cells). When mice were intermittently exposed for 2 weeks, decreased cellularity and CFU-C per tibia were observed at 95 ppm after 6–8 hours/day exposure, whereas at 201 ppm benzene, decreased cellularity (but no effect on CFU-C) was noted after 2 hours/day exposure. A decrease in cellularity and CFU-C was observed after 4–8 hours/day exposure to 201 ppm benzene.

Results of numerous additional studies of laboratory animals (mainly mice, which are particularly sensitive to benzene hematotoxicity, but also rats and pigs) support findings of benzene-induced effects on bone marrow cellularity (hyper- and/or hypocellularity) and colony-forming stem cells, as well as granulocytic hyperplasia, following repeated acute-, intermediate-, or chronic-duration inhalation exposure to benzene vapors at concentrations ranging from 10 to 500 ppm (Baarson and Snyder 1991; Baarson et al. 1984; Chertkov et al. 1992; Corti and Snyder 1996; Cronkite et al. 1982, 1985, 1989; Dempster and Snyder 1991; Dow 1992; Farris et al. 1993, 1997b; Neun et al. 1992, 1994; Plappert et al. 1994a, 1994b; Snyder et al. 1978a, 1980, 1982; Vacha et al. 1990). For example, Dempster and Snyder (1991) observed a 50% reduction in CFU-E (erythroid progenitor cells) in bone marrow of DBA/2 mice exposed to 10 ppm benzene for 6 hours/day for 5 days. Farris et al. (1997b) reported benzene-induced decreased numbers of total bone marrow forming cells, progenitor cells, and differentiating hematopoietic cells in mice exposed to benzene vapor concentrations ≥200 ppm, 6 hours/day, 5 days/week for up to 8 weeks. Replication of primitive progenitor cells in the bone marrow was increased during the exposure period, presumably as compensation for cytotoxicity. Granulocytic hyperplasia was detected in the bone marrow of mice exposed to 300 ppm benzene in air for 6 hours/day, 5 days/week for 16 weeks, and held 18 months after the last exposure (Farris et al. 1993). Prolonged exposure to lower levels had a greater hematotoxic effect than exposure to higher levels for a shorter period of time. Recovery from hematotoxicity has been demonstrated following the cessation of exposure (Cronkite et al. 1982, 1985, 1989) and may be most closely associated with rate of exposure, since longer-term exposure of mice to

316 ppm of benzene caused irreparable hematologic injury, while the hematotoxic effects caused by shorter-term exposure to 10 times higher benzene concentrations were reversible (Cronkite et al. 1989).

One study revealed damaged erythrocytes in the peripheral blood of mice (Luke et al. 1988b). Cytotoxic damage in the bone marrow was dependent on strain and exposure duration. Peripheral blood smears were analyzed weekly from three strains of mice (DBA/2, B6C3F$_1$, and C57BL/6) exposed to 300 ppm benzene for 13 weeks (6 hours/day) for either 5 days/week (Regimen 1) or 3 days/week (Regimen 2). In all three strains, an initial severe depression in rate of erythropoiesis was observed. The return to normal was dependent on strain (Luke et al. 1988b) and regimen (Cronkite et al. 1989; Luke et al. 1988b). An increase in frequency of micronucleated normochromatic erythrocytes (MN-NCE) was observed to be dependent on strain (C57BL/6=B6C3F$_1$>DBA/2) and regimen (Regimen 1 > Regimen 2), whereas the increase in frequency of MN-PCE was dependent on strain (DBA/2>C57BL/6=B6C3F$_1$) but, for the most part, was not dependent on exposure regimen.

Damaged erythroblast-forming cells were also noted in bone marrow (Seidel et al. 1989). A substantial decrease in erythroid colony-forming units and smaller decreases in erythroid burst-forming units occurred in BDF$_1$ mice intermittently exposed to 100 ppm of benzene for 8 weeks (6 hours/day, 5 days/week). Although the effects in the 100 ppm group were not apparent at 8 weeks (the end of experiment), they did occur, and it took over 3 weeks for the erythroid burst-forming units and erythroid colony-forming units to return to their initial values. This reduction in the number of erythroid precursors was reflected by a slight reduction in the number of erythrocytes.

Benzene-induced hematotoxicity was also demonstrated in the spleen of rats and mice following intermediate- or chronic-duration repeated inhalation exposure (Snyder et al. 1978a, 1984; Ward et al. 1985). Snyder et al. (1978a, 1984) reported benzene-induced increased extramedullary hematopoiesis in the spleen. Ward et al. (1985) noted that the finding of hemosiderin in the spleen of benzene-exposed rats could be due to erythrocyte hemolysis.

Musculoskeletal Effects. A case of myelofibrosis was diagnosed in a 46-year-old man in October 1992 (Tondel et al. 1995). The patient worked from 1962 to 1979 as a gasoline station attendant. The patient was referred to the Department of Hematology, University Hospital in Linkoping, Sweden, where a bone marrow biopsy was performed. The patient described symptoms of increasing muscle pain for 1 year, fatigue for 3 weeks, night sweats, and weight loss. A bone marrow biopsy showed myelofibrosis. The TWA concentration for gasoline station attendants was estimated to be <0.2 ppm. The occupational

standard for benzene in Sweden was 0.5 ppm (TWA) and the Swedish short-term exposure limit was 3 ppm. Ruiz et al. (1994) reported musculoskeletal effects in employees from a steel plant of Cubatao, S. Paulo, Brazil, who presented with neutropenia due to benzene exposure. Patients either were employed at the steel plant (mean time of 7 years and 4 months), or were employees of a building construction company working at repairs in the steel plant (mean time of 5 years and 5 months). Sixty percent of the workers had nonspecific clinical complaints such as myalgia.

Hepatic Effects. No specific reports of adverse hepatic effects of inhalation exposure to benzene in humans were found, although Aksoy et al. (1972) reported enlarged livers in workers chronically exposed to benzene at airborne concentrations ranging from 150 to 650 ppm.

CFY rats (20/group) were exposed to pure air, benzene (125 ppm) or benzene (400 ppm) and toluene (265 ppm) for 24 hours/day from gestational day (Gd) 7 through 14 (Tatrai et al. 1980a). The rats were then sacrificed on day 21 of pregnancy. Exposure to 125 ppm benzene caused a slight increase in relative liver weight of 4.67% compared to 4.25% in controls, which was not considered adverse. In a companion study, CFY rats were exposed to continuous benzene inhalation 24 hours/day from day 7 to day 14 of gestation at 0, 47, 141, 470, or 939 ppm atmospheric concentrations (Tatrai et al. 1980b). At 141 ppm benzene, there was a significant increase in relative maternal liver weight.

No treatment-related non-neoplastic histopathological effects on hepatic tissue were found in male Sprague-Dawley rats exposed to 0, 100, or 300 ppm benzene 5 days/week, 6 hours/day for life (Snyder et al. 1978a, 1984) or in AKR/J or C57B1/6J mice similarly exposed to 300 ppm for life (Snyder et al. 1978a, 1980).

Renal Effects. Very little data are available describing renal effects in humans after inhalation exposure to benzene. In a case study involving the death of an 18-year-old boy who intentionally inhaled benzene, the autopsy revealed acute kidney congestion (Winek and Collom 1971). No other details or data were given.

No treatment-related histopathological effects on kidney tissue were found in male Sprague-Dawley rats were exposed to 0, 100, or 300 ppm benzene 5 days/week, 6 hours/day for life (Snyder et al. 1978a, 1984) or in AKR/J or C57B1/6J mice similarly exposed to 300 ppm (Snyder et al. 1978a, 1980).

Dermal Effects. Dermal effects in humans have been reported after acute exposure to benzene vapors (Avis and Hutton 1993). After a fatal occupational exposure to benzene vapors on a chemical cargo ship for only minutes, autopsy reports on three victims revealed hemorrhagic respiratory tissues, and second degree burns on the face, trunk, and limbs (Avis and Hutton 1993). Skin irritation has been noted at occupational exposures of >60 ppm for up to 3 weeks (Midzenski et al. 1992). These effects are due to direct contact of the skin with the vapor, and other dermal effects resulting from direct contact of the skin are discussed in Section 3.2.3.2.

Ocular Effects. Three hundred solvent workers who had inhalation exposures for >1 year to benzene at 33 and 59 ppm for men and women, respectively, complained of eye irritation (Yin et al. 1987b).

Male Charles River CD rats exposed to 0, 1, 10, 30, or 300 ppm benzene 6 hours/day, 5 days/week for 10 weeks exhibited lacrimation at concentrations >10 ppm during the first 3 weeks of treatment (Shell 1980).

These effects are due to direct contact of the eyes with the vapor, and other ocular effects resulting from direct contact of the eyes are discussed in Section 3.2.3.2.

Body Weight Effects. Relatively few studies in animals report changes in body weight after inhalation exposure to benzene. No change in body weight was noted in Sprague-Dawley rats or CD-1 mice exposed to 300 ppm benzene for 13 weeks (Ward et al. 1985). No significant decrease in body weight was observed in CD-1 mice exposed to doses up to 4,862 ppm for 6 hours/day, for 5 days, or at lower doses of 9.6 ppm for 50 days (Green et al. 1981b). Decreases in body weight (15%) were seen in DBA/2 mice after exposure to 300 ppm benzene in air for 6 hours/day, 5 days/week for 2 weeks (Chertkov et al. 1992). Decreased body weight (16–18%) has also been noted in mice exposed to doses of approximately 200 ppm of benzene for 6 hours/day for 7 or 14 days (Aoyama 1986). C57BL mice exhibited a 59% decrease in body weight gain after exposure to 300 ppm benzene, 5 hours/day, 6 days/week over their lifetime (Snyder et al. 1980). Decreased maternal body weight and weight gain have been observed in Sprague-Dawley rats exposed to 50 ppm benzene during gestation days (Gds) 6-15 (Kuna and Kapp 1981), but not in rats exposed to doses up to 300 ppm during premating, mating, gestation, and lactation (Kuna et al. 1992). CFY rats (20/group) were exposed to pure air, benzene (125 ppm), or benzene (400 ppm) plus toluene (265 ppm) for 24 hours/day from Gd 7 through 14 (Tatrai et al. 1980a). The rats were then sacrificed on day 21 of pregnancy. Exposure to 125 ppm benzene caused decreased maternal weight gain (46.74% of starting weight as opposed to 68.82% of starting

weight in controls). Decreased maternal weight gain was also observed in a companion experiment in which the rats were exposed to doses as low as 47 ppm using the same study design (Tatrai et al. 1980b). Decreased maternal body weight was also observed in rats exposed to 2,200 ppm benzene during gestation (Green et al. 1978). Rabbits exposed to 313 ppm benzene on Gd 7–20 exhibited decreased maternal weight gain (Ungvary and Tatrai 1985). Kunming mice exposed to 12.52 ppm benzene for 2 hours/day, 6 days/week for 30 days exhibited no adverse effect on body weight (Li et al. 1992). Sprague-Dawley rats received 100 or 300 ppm benzene vapor for 6 hours/day, 5 days/week for life (Snyder et al. 1978a, 1984). Decreased weight gain, which continued throughout the study, was observed at 30 weeks at 300 ppm, but not at 100 ppm. AKR mice exposed to 100 or 300 ppm and C57BL mice exposed to 300 ppm benzene vapor for 6 hours/day, 5 days/week for life had decreased weight gain at 300 ppm (Snyder et al. 1978a, 1980).

3.2.1.3 Immunological and Lymphoreticular Effects

Immunological effects have been reported in humans with occupational exposure to benzene. There are two types of acquired immunity, humoral and cellular, and benzene damages both. First, benzene has been shown to alter humoral immunity (i.e., to produce changes in levels of antibodies in the blood). Painters who were exposed to benzene (3–7 ppm), toluene, and xylene in the workplace for 1–21 years showed increased serum immunoglobulin values for IgM and decreased values for IgG and IgA (Lange et al. 1973b). The decreased levels of immunoglobulins may represent suppression of immunoglobulin-producing cells by benzene. Other adverse reactions, characterized by a reaction between leukocytes and agglutinins, occurred in 10 of 35 of these workers (Lange et al. 1973a). These results suggest the occurrence of allergic blood dyscrasia in some persons exposed to benzene. However, since the workers were exposed to multiple solvents, the specific role of benzene is uncertain.

The second type of immunity, cellular immunity, is affected by changes in circulating leukocytes and a subcategory of leukocytes, called lymphocytes. Leukopenia was found in a series of studies of workers exposed to benzene at levels ranging from 15 to 210 ppm in various manufacturing processes in Turkey (Aksoy et al. 1971, 1987). Another study also noted signs of preleukemia that included loss of leukocytes and other blood elements, bone marrow histopathology, and enlarged spleens (Aksoy et al. 1972, 1974). Other studies in chronically exposed workers also showed losses of lymphocytes and other blood elements (Cody et al. 1993; Goldwater 1941; Greenburg et al. 1939; Kipen et al. 1989; Ruiz et al. 1994; Yin et al. 1987c). In these studies, benzene levels in workplace air ranged from 1 to 1,060 ppm. Hematological effects reported in these studies are described in Section 3.2.1.2. In one study, routine

leukocyte counts conducted every 3 months on employees of a small-scale industry in China revealed leukopenia in workers exposed to as little as 0.69–140 ppm (mean=6 ppm) for an average period of 5–6 years (Xia et al. 1995). Leukocyte alkaline phosphatase (LAP) activity was increased in benzene workers exposed to about 31 ppm for a chronic time period (Songnian et al. 1982). Increased LAP activity is an indicator of myelofibrosis and is associated with both decreased white blood cell counts and with changes in bone marrow activity. The change in LAP activity could be used in the diagnosis of benzene poisoning since it was more sensitive than the change in the leukocyte count, although it is not a biomarker that is specific for benzene exposure. A study conducted by Li et al. (1994) during 1972–1987 examined 74,828 benzene-exposed workers employed in 672 factories and 35,805 unexposed workers from 109 factories located in 12 cities in China. Estimates of gender-specific rate ratios and a comparison of the rate ratios for females to the rate ratios for males were calculated for the incidence of hematopoietic and lymphoproliferative (HLP) disorders, comparing all exposed workers in each of the occupational groups to unexposed workers. Small increases in relative risks for all HLP disorders for both genders were observed among chemical and rubber manufacturing workers, painters, and paint manufacturers. In another study, an increase in leukocyte count and alkaline phosphatase score was observed in a pipe-fitter who was chronically exposed to 0.9 ppm benzene in addition to other solvents (Froom et al. 1994).

Animal studies support the observations made in humans and show that benzene affects humoral and cellular immunity. A decrease in spleen weight was observed in mice after exposure to benzene at a concentration of 25 ppm, 6 hours/day for 5 days, the same exposure concentration at which a decrease in circulating leukocytes was observed (Wells and Nerland 1991). Benzene decreases the formation of the B-lymphocytes that produce the serum immunoglobulins or antibodies. Exposure to benzene at 10 ppm and above for 6 days decreased the ability of bone marrow cells to produce mature B-lymphocytes in C57BL/6 mice (Rozen et al. 1984). The spleen was also inhibited from forming mature T-lymphocytes at exposure levels of 31 ppm and above. Mitogen-induced blastogenesis of B- and T-lymphocytes was depressed at 10 ppm and above. Peripheral lymphocyte counts were depressed at all levels, whereas erythrocyte counts were depressed only at 100 and 300 ppm. This study is the basis for the acute-duration inhalation MRL of 0.009 ppm (see footnote to Table 3-1 and Appendix A).

A continuation of this line of studies for 6 days to 23 weeks at 300 ppm showed continued decreases in numbers of mature B- and T-lymphocytes produced in the bone marrow, spleen, and thymus (Rozen and Snyder 1985). Abnormalities of humoral and cell-mediated immune responses following benzene exposure are presumably caused by a defect in the lymphoid stem cell precursors of both T- and B-lymphocytes. Bone marrow cellularity increased 3-fold, and the number of thymic T-cells increased

15-fold in benzene-exposed mice between the 6th and the 30th exposure. No corresponding increase in splenic cells was noted. The marked increase in the numbers of cells in bone marrow and thymus was interpreted by the authors to indicate a compensatory proliferation in these cell lines in response to benzene exposure, which may play a role in the carcinogenic response of C57BL mice to inhaled benzene. The lack of response in the spleen suggests a lack of lymphoid restorative capacity in that organ.

Other studies have also shown similar effects on immune functions following inhalation exposure to benzene. These include decreased numbers of circulating leukocytes and decreases in bone marrow cellularity in mice exposed to 100 ppm and higher, 24 hours/day for up to 8 days (Gill et al. 1980); decreased leukocytes and increased leukocyte alkaline phosphatase in rats exposed to 100 ppm for 7 days (Li et al. 1986); decreased leukocytes and bone marrow cellularity in DBA/2 mice exposed to 300 ppm benzene for 2 weeks (Chertkov et al. 1992); decreased leukocytes in mice exposed to 300 ppm for 10 days (Ward et al. 1985); bone marrow hyperplasia, lymphocytopenia, and anemia in mice repeatedly exposed to 100 ppm or higher for a lifetime (Snyder et al. 1978a, 1980); and leukopenia and lymphopenia in rats repeatedly exposed to 100 ppm for life (Snyder et al. 1978a, 1984). Aoyama (1986) noted decreased spleen and thymus weights and decreased levels of B- and T-lymphocytes in blood and spleen in mice exposed 6 hours/day to approximately 48 ppm of benzene for 14 days. Exposures of mice to benzene vapor concentrations ≥100 ppm, 6 hours/day, 5 days/week resulted in significantly reduced numbers of lymphocytes (ranging from 25 to 43% lower than controls) in the spleen, thymus, and femur as early as 1 week following the initial exposure, and persisting throughout an 8-week exposure period (Farris et al. 1997a). In rats, exposure to benzene vapors at a concentration of 400 ppm (but not 200 ppm) for 6 hours/day, 5 days/week for 4 weeks resulted in reduced thymus weight and decreased splenic lymphocytes (Robinson et al. 1997).

Reduced bone marrow cellularity was observed in Swiss Webster and C57BL/6J mice after exposure to 300 ppm benzene for 2 weeks (Neun et al. 1992). Decreased granulopoietic stem cells were observed at 21 ppm in NMRI mice after exposures of 10 days to 2 weeks (Toft et al. 1982). Green and colleagues examined the effect of benzene inhalation on peripheral blood and bone marrow and spleen cells (Green et al. 1981a, 1981b) of CD-1 mice following exposures to a number of regimens. In the acute studies, individual test groups were exposed by inhalation to mean benzene concentrations of 0, 1.1, 9.9, 103, 306, 603, 1,276, 2,416, or 4,862 ppm, 6 hours/day for 5 days. Intermediate studies consisted of two different exposure regimens and concentrations: 6 hours/day, 5 days/week for 10 weeks (50 days) to a mean concentration of 0 or 9.6 ppm benzene; or 6 hours/day, 5 days/week for 26 weeks to a mean concentration

of 0 or 302 ppm benzene. In the acute studies, marrow and splenic cellularities were reduced at 103 ppm. Splenic granulocytes were reduced at all exposure levels except at 9.9 ppm (Green et al. 1981b). The decrease in spleen cellularity correlated with a reduction in spleen weight at all concentrations ≥103 ppm. Mean spleen weights were significantly depressed at 1.1 ppm and at doses above 9.9 ppm, but not at 9.9 ppm. In Green et al. (1981a), marrow concentration of GM-CFU-C was equivalent to or greater than control values at all levels; however, splenic GM-CFU-C concentration was decreased at 103 ppm. Femoral and splenic CFU-S and GM-CFU-C per organ were depressed at 103 ppm. Absolute numbers of GM-CFU-C/femur or spleen were significantly reduced at all higher concentrations. There was no change in the colony/cluster ratio observed in the 5-day experiment. In the 50-day experiments, increased spleen weight, splenic nucleated cellularity, splenic nucleated erythrocytes, and CFU-S were seen at 9.6 ppm (Green et al. 1981a, 1981b). Exposure to 302 ppm for 26 weeks resulted in reduced marrow and spleen cellularity and decreased spleen weight (Green et al. 1981a, 1981b).

Sprague-Dawley SD/Tex rats were exposed to benzene vapor at 0 or 500 ppm for 5 days/week, 6 hours/day for 3 weeks (Dow 1992). In the bone marrow differential counts, rats showed a relative decrease in lymphoid cells at the 500 ppm dose level. There were also decreases in myeloid cells of animals exposed to 500 ppm benzene. In a companion study, purebred Duroc-Jersey pigs were exposed to 0, 20, 100, and 500 ppm benzene vapors 6 hours/day, 5 days/week for 3 weeks (Dow 1992). Exposure to 500 ppm resulted in significant decreases in total white blood cells, T-cells, peripheral blood lymphocytes, and proportion of myeloid cells in bone marrow counts.

Another series of experiments revealed that exposures as low as 25 ppm for 2 weeks caused decreases in the numbers of circulating lymphocytes but did not affect the bone marrow cellularity in C57BL/6 or CBA/Ca mice (Cronkite 1986; Cronkite et al. 1985). Increasing the duration (up to 16 weeks) or concentration (up to 300–400 ppm) produced the same effect of reduced peripheral blood lymphocytes as well as decreased bone marrow cellularity that persisted for up to 8 weeks after exposure (Cronkite et al. 1982, 1985). Similar observations were made in two groups of male CBA/Ca mice exposed to a total of 6,000 ppm of benzene by inhalation using two different regimens (Cronkite et al. 1989). One group was exposed to 316 ppm for a total of 19 times, while the second group was exposed to 3,000 ppm twice. Although both groups had significantly decreased lymphocyte counts, the lymphocyte numbers were much more depressed in the group of mice exposed to 316 ppm of benzene over 19 days. This suggests that, at relatively high exposure levels, repeated or prolonged exposure is more potent than the magnitude of exposure in causing lymphopenia. In both groups, the lymphocyte numbers had not returned to normal

values by 214 days post exposure. The femoral bone marrow cellularity returned to normal levels 32 days after exposure in both groups of mice.

In female BDF_1 mice (C57BL/6xDBA/$2F_1$ hybrids) exposed to 0, 300, or 900 ppm benzene 6 hours/day for 5 days, there was a relative increase in helper lymphocytes (CD4+) at days 3–5, leading to an increase of the T4/T8 (CD4+–CD8+) ratio from 2 in controls to higher values at 8 weeks (Plappert et al. 1994a). No concentration dependency was observed. Granulocytic hyperplasia of the spleen was observed in CBA/Ca mice exposed to 300 ppm benzene for 16 weeks (Farris et al. 1993).

Leukopenia, granulocytopenia, and lymphocytopenia were observed in C57BL/6 mice exposed to 1,000 ppm benzene for 6 hours/day, 5 days/week for up to 6 weeks (Gill et al. 1980). Decreased leukocyte counts were also noted in Sprague-Dawley rats and CD-1 mice exposed to 300 ppm benzene 6 hours/day, 5 days/week for up to 13 weeks (Ward et al. 1985). In mice, the most common compound-related histopathological findings were splenic periarteriolar lymphoid sheath depletion, lymphoid depletion in the mesenteric lymph node, and plasma cell infiltration of the mandibular lymph node. Exposure to the highest concentration caused a decrease in leukocyte and lymphocyte counts in Sprague-Dawley rats. Hematological changes at 300 ppm were accounted for by decreased leukocyte counts in males on day 14 and in females on day 91. Decreases in percentage of lymphocytes in males and females started on day 14 and lasted through day 91. Rats exhibited decreased femoral marrow cellularity as the only histological change. Treatment-related changes were not observed at lower concentrations. Rabbits, rats, and guinea pigs exposed to 80–88 ppm for 32–269 days also had decreased leukocyte counts (Wolf et al. 1956). Leukocyte alkaline phosphatase values were increased and leukocyte counts were decreased in rats exposed to 4,570 ppm for 20 weeks (Songnian et al. 1982). Exposure of C57BL mice to 10 ppm benzene for 6 hours/day, 5 days/week, for 24 weeks caused depressions in the numbers of splenic nucleated red cells and lymphocytes (Baarson et al. 1984).

Li et al. (1992) observed a 26% decrease in spleen weight in male Kunming mice exposed to 12.52 ppm benzene 2 hours/day, 6 days/week for 30 days. Examination of the bone marrow showed decreases in myelocytes, premyelocytes, myeloblasts, and metamyeloblasts at the same dose level.

Benzene also affects functional immune responses, as indicated by decreased resistance to infectious agents. Pre-exposure to benzene at 30 ppm for 5–12 days increased the bacterial counts in mice on day 4 of infection with *Listeria monocytogenes* (Rosenthal and Snyder 1985). Recovery of the immune system was noted on day 7. The effects did not occur at 10 ppm. In addition, a concentration-dependent

statistically significant depression was noted in T- and B-lymphocyte populations from day 1 through day 7 at 30 ppm and above. B-cells were more sensitive to benzene than were T-cells on a percentage-of-control basis. These results indicate a benzene-induced delay in immune response to *L. monocytogenes*. Concentrations of 200 or 400 ppm for 4–5 weeks (5 days/week) suppressed the primary antibody response to tetanus toxin in mice, but there was no effect at 50 ppm (Stoner et al. 1981). In another intermediate-duration exposure study, no changes were noted in the numbers of splenic B-cells, T-cells, or T-cell subsets in C57BL/6 mice exposed to 100 ppm of benzene 5 days/week for 20 days (Rosenthal and Snyder 1987). However, when splenic T-cells from mice treated with 10 ppm and 100 ppm were tested *in vitro* for their capacity to respond to foreign antigens (alloantigens) in the mixed lymphocyte reaction (MLR), the MLR response was delayed. Further analysis showing that this delayed MLR response was not due to the presence of benzene-induced suppressor cells and indicated that benzene impaired the functional abilities of alloreactive T-cells. This study is the basis for the intermediate-duration inhalation MRL of 0.006 ppm (see footnote to Table 3-1 and Appendix A). A similar *in vitro* observation was made using T-cells from mice exposed to 100 ppm of benzene 5 days/week for 3 weeks. These cells had a reduced tumor cytolytic activity, suggesting a benzene-induced impairment of cell-mediated immunity. The impaired cell-mediated immune function was also apparent *in vivo*. Mice exposed to 100 ppm for a total of 100 days were challenged with 10,000 polyoma virus-induced tumor cells (PYB6), and 9 of 10 mice had reduced tumor resistance and developed tumors that were lethal (Rosenthal and Snyder 1987).

The highest NOAEL values and all reliable LOAEL values for immunological effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.4 Neurological Effects

Following acute inhalation of benzene, humans exhibit symptoms indicative of central nervous system effects (Cronin 1924; Flury 1928; Greenburg 1926; Midzenski et al. 1992). These symptoms, reported to occur at levels ranging from 300 to 3,000 ppm, include drowsiness, dizziness, headache, vertigo, tremor, delirium, and loss of consciousness. Acute exposure (5–10 minutes) to higher concentrations of benzene (approximately 20,000 ppm) can result in death, which has been associated with vascular congestion in the brain (Avis and Hutton 1993; Flury et al. 1928). Lethal exposures are also associated with nonspecific neurological symptoms similar to those reported for nonlethal exposures. These symptoms are similar to the consequences of exposure to multiple organic solvents and are reversible when symptomatic workers are removed from the problem area (Kraut et al. 1988; Yin et al. 1987b). In reports

of cases of benzene poisoning, subjects exhibited headaches, nausea, tremor, convulsions, and unconsciousness, among other neurological effects (Cronin 1924; Greenburg 1926; Midzenski et al. 1992; Tauber 1970).

Chronic exposure to benzene has been reported to produce neurological abnormalities in humans. Of eight patients (six with aplastic anemia and two with preleukemia) with previous occupational exposure to adhesives and solutions containing 9–88% benzene, four of the six patients with aplastic anemia showed neurological abnormalities (global atrophy of lower extremities and distal neuropathy of upper extremities) (Baslo and Aksoy 1982). Air concentrations in the workplace were reported to have reached levels of ≥210 ppm. These findings suggest that benzene may induce toxic effects on the nervous system involving peripheral nerves and/or spinal cord. The limitations of this study are that benzene exposure levels were not monitored and that there was a possibility of an additional exposure to toluene (6.37–9.25%).

Chronic exposure to benzene and toluene was studied in 121 workers exposed to benzene for 2–9 years (Kahn and Muzyka 1973). The air concentration of benzene between 1962 and 1965 was 6–15.6 ppm (20–50 mg/m^3), while the toluene vapors did not exceed the 5 mg/m^3 level. Subsequently (the authors do not specify when), the air levels of both benzene and toluene have not exceeded the 5 mg/m^3 level. Seventy-four of the examined workers complained of frequent headaches (usually at the end of the work day), became tired easily, had difficulties sleeping, and complained of memory loss. The limitations of this study are that workers were exposed to both benzene and toluene and that the precise dose and duration of exposure are not known.

Tondel et al. (1995) reported the case of a gasoline station attendant who had worked from 1962 to 1979. The patient described symptoms of fatigue for 3 weeks and night sweats, among other symptoms.

The neurotoxicity of benzene has not been studied extensively in animals. Female Sprague-Dawley rats exhibited lethargy after exposure to 2,200 ppm benzene, but not 300 ppm, on Gd 6–15 (Green et al. 1978). Male albino SPF rats from a Wistar-derived strain exposed to benzene for 4 hours in glass chambers (dose not specified) exhibited depression of evoked electrical activity in the brain; the authors calculated the 30% effect level (depressed activity) as 929 ppm (Frantik et al. 1994). When female H strain mice were exposed to benzene for 2 hours, the 30% effect level for depression of evoked electrical activity in the brain was 856 ppm (Frantik et al. 1994). In rabbits, symptoms that occurred 3.7 minutes following acute exposure to benzene at 45,000 ppm were relaxation and light narcosis

(Carpenter et al. 1944). As the time after exposure progressed, so did the symptoms to include excitation, chewing, and tremors (after 5 minutes), loss of pupillary reflex to strong light (after 6.5 minutes), loss of blinking reflex (after 11.4 minutes), pupillary contraction (after 12 minutes), and involuntary blinking (after 15.6 minutes). Behavioral tests of C57BL/6 mice showed significant increase in licking of sweetened milk after 1 week of exposure to 300 ppm; a 90% decrease in hind limb grip strength after one exposure to 1,000 or 3,000 ppm (data for 100 ppm were not reported); and tremors after one exposure to 3,000 ppm that subsided 30 minutes after the exposure (Dempster et al. 1984). In another study, designed to reflect occupational exposure, male CD-1 and C57BL/6 mice were exposed to 300 or 900 ppm of benzene 6 hours/day for 5 days followed by 2 weeks of no exposure after which the exposure regimen was repeated for an unspecified amount of time (Evans et al. 1981). The following seven categories of behavioral activities were monitored in exposed and control animals: stereotypic behavior, sleeping, resting, grooming, eating, locomotion, and fighting. Only minimal and insignificant differences were observed between the two strains of mice. Increased behavioral activity was observed after exposure to benzene in both strains of mice. Mice exposed to 300 ppm of benzene had a greater increase than those exposed to 900 ppm, probably because of narcosis-like effects induced at the higher exposure level (Evans et al. 1981). It is not known if benzene induces behavioral changes by directly acting upon the central nervous system. It is also not known whether these changes occur before or after hematological changes.

Li et al. (1992) exposed male Kunming mice to 0, 0.78, 3.13, or 12.52 ppm benzene for 2 hours/day, 6 days/week for 30 days, and then monitored brain and blood acetylcholinesterase, forelimb grip strength, locomotor activity, and rapid response. Significantly increased grip strength was observed at 0.78 ppm, whereas at the higher doses, grip strength decreased significantly. Rapid response showed a significant increase at the low dose; the two higher doses showed a significant depressed rapid response. Locomotor activity increased at the low dose, was similar to control values at the middle dose, and decreased at the high dose. However, these changes were not significantly different from the control values. A significant decrease in acetylcholinesterase activity was noted in the brain, but it was not large enough to be considered adverse; no change in acetylcholinesterase levels in the blood was observed. However, the exposure levels used by Li et al. (1992) were more than 10-fold lower than those eliciting signs of neurotoxicity in other animal studies, an indication that the actual exposure levels in the study of Li et al. (1992) may have been higher than indicated. Uncertainty regarding actual exposure levels renders this study of little value for purposes of risk assessment.

All reliable LOAEL values for neurological effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.5 Reproductive Effects

Data on the reproductive effects of occupational exposure to benzene suggest that benzene may impair fertility in women (Mukhametova and Vozovaya 1972; Vara and Kinnunen 1946). However, the findings are inconclusive due to uncertainties in exposure assessment and the limited data collected. In one study, 30 women with symptoms of benzene toxicity were examined (Vara and Kinnunen 1946). The levels of benzene in air were not specified, but are assumed to have been much greater than the 1 ppm permitted in today's working environment. Twelve of these women had menstrual disorders (profuse or scanty blood flow and dysmenorrhea). Ten of the 12 women provided information on fertility. Of these 10 women, 2 had spontaneous abortions, and no births occurred during their employment, even though no contraceptive measures had been taken. This led the investigators to suggest that benzene has a detrimental effect on fertility at high levels of exposure. However, the study failed to provide verification that the absence of birth was due to infertility. Gynecological examinations revealed that the scanty menstruations of five of the patients were due to ovarian atrophy. This study is limited in that an appropriate comparison population was not identified. Additionally, little follow-up was conducted on the 30 women with regard to their continued work history and possible symptoms of benzene toxicity.

Disturbances of the menstrual cycle were found in women workers exposed to aromatic hydrocarbons (benzene, toluene, xylene) (Michon 1965). The exposure levels of benzene and toluene were below 0.25 ppm. The observed group consisted of 500 women, 20–40 years old. One hundred controls were included in the study. The results showed that 21% of exposed women whose work involved sitting or standing had irregular menstrual cycles compared to 12% in the control group. Brief (up to 2 days), long (6–9 days), and prolonged (over 9 days) menstrual cycles were present in 26% of women who performed lifting during their work as compared to 13% in the control group. Irregular amounts of menstrual flow and pain were also observed in female workers exposed to aromatic hydrocarbons. The major limitations of this study are that the exposure occurred from a mixture of chemicals, levels of exposure were not well defined, duration of exposure was not stated, and activities of the controls were not provided.

Another study examined the reproductive function and incidence of gynecological effects in 360 women exposed to petroleum (a major source of benzene) and chlorinated hydrocarbons both dermally and by inhalation (Mukhametova and Vozovaya 1972). However, dermal exposure was considered to be

negligible. The concentrations of benzene in the air were not well documented. When compared to female workers with no chemical exposure, female gluers had developed functional disturbances of the menstrual cycle. Additionally, as chemical exposure time increased, there were increases in the number of premature interruptions of pregnancy, the percentage of cases in which the membranes ruptured late, and the number of cases of intrauterine asphyxia of the fetus. The study limitations (including lack of exposure history, simultaneous exposure to other substances, and lack of follow-up) make it difficult to assess the effects of benzene on reproduction.

Reproductive competence of male workers in two organic chemical factories in France was evaluated by Stucker et al. (1994). Analysis of 1,739 pregnancies that ended in spontaneous abortion or birth was presented. Paternal exposure to benzene for each pregnancy was described as exposure in the 3 months immediately before conception, and as previous job exposures. Benzene exposure was graded at two levels: <5 ppm (low) and ≥5 ppm (moderate). Of the 1,739 pregnancies described, 171 ended in a spontaneous abortion (rate=9.8%). According to exposure categories, 1,277 pregnancies were defined as non-exposed (mate not exposed) and 270 whose mates were exposed at some time before conception. For the 270 pregnant women, 145 of their mates were exposed during the 3 months immediately preceding conception. The frequency of spontaneous abortion was not significantly higher for the paternal group exposed at any time before conception than in the non-exposed group; nor was it higher for the group exposed during the 3 months immediately before conception.

In CFY rats exposed to either pure air or benzene (125 ppm) for 24 hours/day from Gd 7 through 14, no effect on implantation number was observed (Tatrai et al. 1980a). No changes in maternal body weight were observed in Sprague-Dawley rats exposed to 100 ppm benzene for 6 hours/day on Gd 6–15 (Coate et al. 1984). Pregnant rabbits exposed 12 hours/day to 156.5 or 313 ppm benzene on Gd 7–20 showed an increase in the number of abortions and resorptions at 312 ppm (Ungvary and Tatrai 1985). However, in other developmental toxicity studies, no effect on the number of resorptions was seen in rats at doses as high as 2,200 ppm (Green et al. 1978), in mice at 500 ppm (Murray et al. 1979), or in rabbits at doses of 500 ppm (Murray et al. 1979).

Reproductive effects have been noted in experimental animals exposed for intermediate durations, but the levels of exposure were higher (80–6,600 ppm) than those to which humans are exposed in the modern industrial environment (Ward et al. 1985; Wolf et al. 1956). In an intermediate-duration inhalation study, groups of male and female CD-1 mice were exposed to benzene vapor concentrations of 0, 1, 10, 30, or 300 ppm, 5 days/week, 6 hours/day to benzene vapor for 13 weeks (Ward et al. 1985). Histopathological

changes were observed in ovaries (bilateral cysts) and testes (atrophy/degeneration, decrease in spermatozoa, moderate increase in abnormal sperm forms) of mice exposed to 300 ppm benzene; the severity of gonadal lesions was greater in the males. An inhalation study was conducted exposing rats (6,600 ppm), rabbits (80 ppm), and guinea pigs (88 ppm) to benzene for 7–8 hours/day, 5 days/week for 93, 243, and 32 or 269 days, respectively (Wolf et al. 1956). Male rats showed an increase in testicular weight after 93 days at the 6,600 ppm level. The guinea pigs showed a slight increase in average testicular weight at the 88 ppm level. Rabbits showed slight histopathological testicular changes (degeneration of the germinal epithelium) when exposed to 80 ppm. Since only one or two rabbits were used in this study, it was not possible to draw any conclusions regarding benzene's ability to induce testicular damage in the rabbits. Continuous exposure of female rats to 210 ppm benzene for 10–15 days before cohabitation with males and 3 weeks after cohabitation resulted in a complete absence of litters (Gofmekler 1968). It is not known whether this was due to failure to mate, infertility, or early preimplantation losses of fertilized ova. In a fertility study, female rats exposed up to 300 ppm benzene for 10 weeks during premating, mating, gestation, and lactation showed no effect on indices of fertility, reproduction, and lactation (Kuna et al. 1992).

The highest NOAEL values and all reliable LOAEL values for reproductive effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.6 Developmental Effects

The available human data on the developmental effects of benzene after inhalation exposure are inconclusive. The studies designed specifically to investigate developmental effects are limited, primarily because of concomitant exposure to other chemicals, inadequate sample size, and lack of quantification of exposure levels (Budnick et al. 1984; Goldman et al. 1985; Heath 1983; Olsen 1983). Benzene crosses the human placenta and is present in the cord blood in amounts equal to or greater than those in maternal blood (Dowty et al. 1976). In a study of subjects with known benzene poisoning in Italy, Forni et al. (1971a) reported the case of one pregnant worker exposed to benzene in the air during her entire pregnancy. Although she had severe pancytopenia and increased chromosomal aberrations, she delivered a healthy son with no evidence of chromosomal alterations. The following year, she delivered a healthy daughter. However, increased frequency of chromatid and isochromatid breaks and sister chromatid exchange was found in lymphocytes from 14 children of female workers exposed by inhalation to benzene (dose not specified) and other organic solvents during pregnancy (Funes-Cravioto et al. 1977).

No mention was made of whether the mothers showed signs of toxicity or whether physical abnormalities occurred among their offspring.

There are numerous inhalation studies in which animals have been exposed to benzene during pregnancy (Coate et al. 1984; Green et al. 1978; Kuna and Kapp 1981; Murray et al. 1979; Tatrai et al. 1980a, 1980b; Ungvary and Tatrai 1985). None of these studies demonstrated that benzene was teratogenic even at levels that induced maternal and fetal toxicity. Fetotoxicity was evidenced by decreased body weight and by increased skeletal variants such as missing sternebrae and extra ribs, which were not considered to be malformations. Alterations in hematopoiesis have also been observed in the fetuses and offspring of pregnant mice exposed to low levels of benzene (Keller and Snyder 1986, 1988). These studies are discussed below.

Mice exposed to 500 ppm benzene for 7 hours/day on days 6–15 of pregnancy exhibited fetal growth retardation (i.e., decreased fetal body weight) and increased minor skeletal variants (i.e., delayed ossification) (Murray et al. 1979). There were no fetal malformations and no significant effect on the incidence of pregnancy, average number of live fetuses, resorptions per litter, or maternal weight gain. No malformations in fetuses and no significant effects on incidence of pregnancy, average number of live fetuses, or resorptions per litter were observed when rabbits were exposed to benzene 7 hours/day at 500 ppm during gestation for 9 days, although an increase in minor skeletal variations was observed (Murray et al. 1979).

Pregnant mice exposed 12 hours/day to 156.5 or 313 ppm benzene on Gd 6–15 had pups with significant weight retardation and retardation of skeletal development, but no malformations (Ungvary and Tatrai 1985). A parallel study in rabbits showed that inhalation of benzene at 313 ppm caused fetal weight reduction, and an increase in minor fetal anomalies (Ungvary and Tatrai 1985).

As was the case with mice and rabbits, the fetotoxicity of benzene in rats is also demonstrated by retarded fetal weight and/or minor skeletal variants (Coate et al. 1984; Green et al. 1978; Kuna and Kapp 1981; Tatrai et al. 1980b). In an experiment conducted by Green et al. (1978), pregnant Sprague-Dawley rats were exposed to 100, 300, or 2,200 ppm benzene for 6 hours/day on Gd 6–15. Exposure to high levels of benzene (2,200 ppm) during gestation resulted in a significant decrease in fetal weight, whereas dams breathing air containing lower levels of benzene (100 or 300 ppm) during gestation bore young that were similar in weight and crown–rump length to control pups. Statistically significant numbers of fetuses with delayed ossification were found in groups exposed to concentrations of 300 and 2,200 ppm. The

litter incidence of missing sternebrae was significantly increased in the 100 and 2,200 ppm exposure groups. Maternal toxicity, as indicated by a decrease in maternal weight gain, was evident only at the 2,200 ppm level. The female offspring appeared to be affected to a greater extent than males with respect to delayed ossification and missing sternebrae.

Kuna and Kapp (1981) found decreased fetal weight after exposure to 50 ppm. From a group of 151 pups examined after *in utero* exposure to 500 ppm benzene, a single pup exhibited exencephaly. In the same study, of 98 pups examined for skeletal effects after *in utero* exposures of 500 ppm, a single pup had angulated ribs and 2 others had nonsequential ossification of the forefeet. These anomalies were not statistically significant and may have resulted from maternal nutritional stress.

No significant skeletal malformations occurred in pups of rats exposed during gestation to 47 ppm for 8 days, 24 hours/day (Tatrai et al. 1980b) or 100 ppm for 10 days, 6 hours/day (Coate et al. 1984). Decreased fetal weights were seen at 47 ppm (Tatrai et al. 1980b) and 100 ppm (Coate et al. 1984), and increased fetal mortality was observed at 141 ppm (Tatrai et al. 1980b). In CFY rats exposed to pure air or 125 ppm benzene on Gd 7–14, there was a 17% decrease in placental weight, a decrease in mean fetal weight, and evidence of skeletal retardation (Tatrai et al. 1980a). Continuous exposure of female rats to 6 concentrations of benzene ranging from 0.3 to 210 ppm for 10–15 days before cohabitation with males and 3 weeks after did not affect newborn weight or induce malformations, but there were differences in the weights of individual organs of the dams at all exposure levels (Gofmekler 1968). There was a slight tendency toward decreased litter sizes at 20 ppm of benzene. A complete absence of litters resulted from exposure to 210 ppm. It is not known whether this was due to failure to mate, infertility, or early preimplantation losses of fertilized ova.

Alterations in hematopoiesis have also been observed in the fetuses and offspring of pregnant mice exposed to benzene (Keller and Snyder 1986). Administration of 20 ppm benzene to pregnant Swiss Webster mice for 6 hours/day on Gd 6–15 caused reductions in the levels of the CFU-E of the fetuses, whereas 5 and 10 ppm benzene caused enhancement of these colony-forming cells. In 2-day-old neonates, CFU-E numbers in the 5 ppm group returned to control values, but the 10 ppm neonates showed a bimodal response by litter. Granulocytic colony-forming cells were enhanced in neonates exposed *in utero* to 20 ppm benzene. Some of the mice exposed to 10 ppm prenatally were re-exposed to 10 ppm as adults. Their hematopoietic progenitor cell numbers were depressed compared with controls exposed for the first time as adults. No tests were conducted on the dams after benzene exposure.

In a follow-up study, pregnant Swiss Webster mice were exposed 6 hours/day on Gd 6–15 to 5, 10, or 20 ppm benzene (Keller and Snyder 1988). The results indicated that 16-day fetuses, when checked for erythrocyte and leukocyte counts, hemoglobin analysis, and the proliferating pool of differentiating hematopoietic cells, had no noteworthy change at any of the exposure levels. In contrast, 2-day neonates exposed *in utero* to all concentrations of benzene exhibited a reduced number of circulating erythroid precursor cells and, at 20 ppm, had increased numbers of hepatic hematopoietic blast cells and granulopoietic precursor cells accompanied by decreased numbers of erythropoietic precursor cells. Six-week-old adult mice exposed *in utero* to 20 ppm of benzene had a similar pattern of enhanced granulopoiesis. However, this effect was not clearly evident in 6-week-old adult mice exposed *in utero* to 5 or 10 ppm.

The results of inhalation studies conducted in experimental animals have been fairly consistent across species. It has been suggested that benzene fetotoxicity in animals is a function of maternal toxicity because the joint occurrence of a decrease in fetal weight and an increase in skeletal variants usually occurs when there is a decrease in maternal weight (Tatrai et al. 1980b). However, the mechanism underlying developmental toxicity has not been fully elucidated, and there are few data on the effect of benzene on maternal food consumption and on blood levels of benzene and its metabolites in the dams and their fetuses. There are apparently none of the usual fetotoxic findings after exposure *in utero* to low concentrations of benzene (10 ppm) (Coate et al. 1984; Kuna and Kapp 1981). As stated above, there is evidence for persistent hematopoietic anomalies in animals exposed *in utero* to benzene at 20 ppm (Keller and Snyder 1988). They may also exist at lower concentrations, but adequate testing has not been performed.

The highest NOAEL value and all reliable LOAEL values for developmental effects in each species following acute exposure are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.7 Cancer

Epidemiological studies and case reports provide clear evidence of a causal relationship between occupational exposure to benzene and benzene-containing solvents and the occurrence of acute nonlymphocytic leukemia (ANLL), particularly the myeloid cell type (acute myelogenous leukemia, AML) (EPA 1985b, 1998; Hayes et al. 1997; IARC 1982, 1987; IRIS 2007; Rinsky et al. 1987, 2002; Yin et al. 1996a, 1996b). Some of the studies also provide suggestive evidence of associations between benzene exposure and non-Hodgkin’s lymphoma (NHL) and multiple myeloma (Hayes et al. 1997;

Rinsky et al. 1987). The epidemiological studies are generally limited by confounding chemical exposures and methodological problems, including inadequate or lack of exposure monitoring and low statistical power (due to small numbers of cases), but a consistent excess risk of leukemia across studies indicates that benzene is the causal factor. Many of the earlier studies are additionally limited by a lack of information on leukemia cell types other than AML, because leukemia used to be considered a single diagnostic category for epidemiological purposes, due in part to historical nomenclature, small numbers of deaths by cell type, and unavailability of cell-type-specific rates for comparison.

Two series of studies on workers exposed to benzene in Ohio (the Pliofilm study) (e.g., Rinsky et al. 1981, 1987, 2002) and China (the NCI/CAPM study) (e.g., Hayes et al. 1997; Yin et al. 1996a, 1996b) have yielded particularly strong data on the leukemogenic potential of benzene and are summarized below. The studies of the Pliofilm cohort provide the best set of data for evaluating human cancer risks from benzene exposure because, in comparison to other published studies, the Pliofilm workers had the fewest reported co-exposures to other potentially carcinogenic substances and experienced a greater range of estimated exposures to benzene (EPA 1998). The NCI/CAPM Chinese study is one of the largest of its type ever undertaken and evaluated many thousands of benzene-exposed workers, enabling detection of significantly elevated risks at unusually low levels of exposure.

A number of studies were performed on a cohort of workers exposed to benzene in three rubber hydrochloride (‘Pliofilm’) manufacturing plants in Ohio (Collins et al. 1997; Finkelstein 2000; Infante 1978; Infante et al. 1977; Ireland et al. 1997; Paxton et al. 1994a, 1994b; Rinsky et al. 1981, 1987, 2002; Schnatter et al. 1996a; Wong 1995). Analyses using various expansions and updates of the cohort, statistical methods, and exposure estimates have consistently shown a significant relationship between exposure to benzene and excess mortality from leukemia. In the report by Rinsky et al. (1987), a cohort of 1,165 white males employed between 1940 and 1965 and followed through 1981 experienced increased mortality from all leukemias (9 observed versus 2.7 expected; standardized mortality ratio [SMR]=3.37, 95% CI 1.54–6.41) and multiple myeloma (4 observed versus 1 expected; SMR=4.09, 95% CI 1.10–10.47). Death rates for age-matched U.S. white males during the same calendar period were used for comparison with death rates observed in the cohort. Assessment after an additional 15 years of follow-up showed declines in the SMRs for both leukemias (2.56, 95% CI 1.43–4.22) and multiple myeloma (2.12, 95% CI 0.69–4.96), suggesting that the excess risks diminished with time since exposure (Rinsky et al. 2002). Exposures in the most recent 10 years were most strongly associated with leukemia risk, and there was no significant relation between leukemia death and benzene exposures received more than 20 years previously (Finkelstein 2000). AML accounted for most of the increased leukemia

(SMR=5.03, 95% CI 1.84–10.97) (Schnatter et al. 1996a; Wong 1995). The risk of all leukemias and AML, but not multiple myeloma, significantly increased with increasing cumulative exposure above 200 ppm-years (Rinsky et al. 1987, 2002; Wong 1995). Analysis of 4,417 workers from one of the Pliofilm plants showed no clear evidence that the risks of all leukemias, ANLL, multiple myeloma, or other lymphohematopoietic cancers increased with increasing exposure at lower levels of cumulative exposure (1–72 ppm-years), and the number of peak exposures over 100 ppm for ≥40 days was a better predictor of risk for all leukemias and multiple myeloma (Collins et al. 2003; Ireland et al. 1997). No clear associations between exposure to benzene and mortality from NHL or Hodgkin's disease were reported (Collins et al. 2003; Ireland et al. 1997; Rinsky et al. 2002).

A collaborative study between the National Cancer Institute and the Chinese Academy of Preventive Medicine (NCI/CAPM) evaluated incidence rates (occurrence of disease and cause of death) for lymphohematopoietic malignancies and other hematologic disorders in a cohort of 74,828 benzene-exposed and 35,805 nonexposed workers employed in 672 factories in 12 cities in China (Hayes et al. 1996, 1997, 2001; Linet et al. 1996). The joint NCI/CAPM study is an expansion of earlier studies performed by CAPM alone (Yin et al. 1987a, 1987b, 1987c, 1989). The workers were employed from 1972–1987, followed for an average of nearly 12 years, and worked in various job categories using benzene as a solvent for paints, varnishes, glues, coatings, and other products. The derivation of the cohort from many different factories and job types suggests that the members were concurrently exposed to many other chemicals. Findings in the exposed workers included significantly increased relative risk (RR) for all hematologic neoplasms (RR=2.6, 95% CI 1.4–4.7), all leukemias (RR=2.5, 95% CI 1.2–5.1), ANLL (RR=3.0, 95% CI 1.0–8.9), and combined ANLL and precursor myelodysplastic syndromes (ANLL/MDS; RR=4.1, 95% CI 1.4–11.6) (Hayes et al. 1997). Increases were also observed for other (unspecified) leukemias (RR=2.0, 95% CI=0.7–5.4), but the results were not statistically significant. Analysis by level of average benzene exposure (<10, 10–24, and ≥25 ppm) and cumulative exposure (<40, 40–99, and ≥100 ppm-years) indicated that the risk for all hematologic neoplasms was significantly increased at <10 ppm (RR=2.2, 95% CI 1.1–4.2) and <40 ppm-years (RR=2.2, 95% CI 1.1–4.5), respectively. The risks for all leukemias, ANLL, and ANLL/MDS were increased at exposure levels of 10–24 ppm (average) and 40–99 ppm-years (cumulative). The elevated risks showed weak tendencies to increase with increasing average and cumulative levels of exposure. Analysis by duration of exposure (<5, 5–9, or ≥10 years) did not show increased risk with increasing exposure duration. Analysis by occupational group (coatings, rubber, chemical, shoe, other/mixed) showed that the increased risks for ANLL and ANLL/MDS were consistent across the spectrum of industries studied, implying that the associations were due to the common exposure to benzene rather than other industry-specific exposures.

The results of a study of shoe factory workers in Italy are similar to those of the Pliofilm and NCI/CAPM studies in showing that the risk of leukemia increases with increasing exposure to benzene (Costantini et al. 2003; Paci et al. 1989). The cohort was followed from 1950 to 1999 and consisted of 891 men and 796 women who were exposed to estimated benzene concentrations ranging from 0 to 92 ppm, and had mean cumulative exposures of 71.8 and 43.4 ppm-years, respectively (exposure durations not reported). Leukemia risk was significantly increased in both sexes in the highest of four exposure categories and most apparent in the men. For cumulative exposures of <40, 40–99, 100–199, and >200 ppm-years, the leukemia SMR values for the men were 1.4 (95% CI 0.2–5.0), 3.7 (95% CI 0.1–20.6), 3.0 (95% CI 0.4–10.9), and 7.0 (95% CI 1.9–18.0), respectively. Leukemia subtypes were not evaluated. The findings are consistent with earlier epidemiologic studies and case reports showing increased incidences of leukemia in shoe factory and rotogravure plant workers exposed to high benzene levels during its use as a solvent (Aksoy et al. 1974, 1987; IARC 1982; Vigliani and Forni 1976).

No significant increases in leukemia were found in chemical industry workers (Bloemen et al. 2004; Bond et al. 1986; Ott et al. 1978) or petroleum industry workers (Lewis et al. 1997; Raabe and Wong 1996; Rushton and Romaniuk 1997; Schnatter et al. 1993, 1996b, 1996c; Tsai et al. 1983) exposed to lower levels of benzene. Cause-specific mortality was determined in a prospective study of 2,266 chemical workers who were exposed to benzene in various Dow Chemical Company manufacturing processes between 1938 and 1970 (Bloemen et al. 2004). The workers were followed from 1940–1996 and had an average duration of exposure, intensity of exposure, and cumulative exposure of 4.8 years, 9.6 ppm, and 39.7 ppm-years, respectively. There were no significant increases in risk for any lymphohematopoietic malignancies, including all leukemias, ANLL, chronic lymphatic leukemia (CLL), NHL, and multiple myeloma. A previous investigation of this cohort found a significantly increased risk of ‘myelogenous’ leukemias (SMR=4.44, 95% CI not reported) (Bond et al. 1986; Ott et al. 1978), but this result is less reliable than those of the current study because it is based on a much smaller number of deaths.

A meta-analysis was conducted on 19 cohorts of petroleum workers in the United States and the United Kingdom that were pooled into a single database for cell-type-specific leukemia analysis (Raabe and Wong 1996). The combined cohort consisted of 208,741 workers, mainly refinery employees who contributed more than 4.6 million person-years of observation. Benzene exposures were mainly from handling gasoline and the estimated mean and cumulative exposures for the most exposed jobs were <1 ppm and <45 ppm-years, respectively. No increased risks were found for mortality from AML, chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), or CLL. Analyses limited to

studies of refinery workers or studies with at least 15 years of follow-up yielded similar results. The negative results of this meta-analysis are consistent with those of case-control studies of the common leukemia cell types in petroleum workers (Rushton and Romaniuk 1997; Schnatter et al. 1993, 1996b, 1996c). A case-control study of hairy cell leukemia, a rare B-lymphoid chronic leukemia, found no association between reported exposure to benzene and the risk of this cancer in workers from various occupations with generally low levels of exposure (Clavel et al. 1996).

A possible association between occupational exposure to benzene and NHL is suggested by results of the most recent analysis (Hayes et al. 1997) of the cohort from the NCI/CAPM Chinese study summarized above. The relative risk for mortality from NHL in the whole cohort was 3.0 (95% CI 0.9–10.5), an increase that was not statistically significant. However, the risk for NHL did significantly increase at the highest level and duration of benzene exposure. For exposure to average concentrations of <10, 10–24, and ≥25 ppm, the RR values were 2.7 (95% CI 0.7–10.6), 1.7 (95% CI 0.3–10.2), and 4.7 (95% CI 1.2–18.1), respectively (P for trend=0.04). For cumulative exposures of <40, 40–99, and ≥100 ppm-years, the RR values were 3.3 (95% CI 0.8–13.1), 1.1 (95% CI 0.1–11.1), and 3.5 (95% CI 0.9–13.2), respectively (P for trend=0.02). Additionally, the risk for NHL significantly increased with increasing duration of exposure among workers exposed for <5 years (RR=0.7, 95% CI 0.1–7.2), 5–9 years (RR=3.3, 95% CI 0.7–14.7), and >10 years (RR=4.2, 95% CI 1.1–15.9) (P for trend=0.01).

Although the results of the NCI/CAPM Chinese study (Hayes et al. 1997) indicated a possible association between exposure to benzene and NHL, other cohort mortality studies found no significant increases in NHL mortality. These studies include the entire Pliofilm cohort (Rinsky et al. 2002); a total of 4,417 workers from one of the three Pliofilm plants assessed for cumulative exposures of 0, <1, 1–6, and >6 ppm-years (Collins et al. 2003); a cohort of 2,266 chemical manufacturing workers exposed to a cumulative benzene level of 39.7 ppm-years (Bloemen et al. 2004); and a meta-analysis of 26 cohorts of petroleum workers from the United States and five other countries with expected low exposures to benzene (Wong and Raabe 2000). Furthermore, case-control studies provide no indications of an association between benzene exposure and risk of NHL (Schnatter et al. 1996b; Wong and Raabe 2000), and the adequacy of the data from the NCI/CAPM Chinese study (Hayes et al. 1997) has been questioned (Dosemeci et al. 1994; Wong 1999; Wong and Raabe 2000).

Other evidence suggests a possible association between occupational exposure to benzene and increased risk of multiple myeloma. Mortality from multiple myeloma was initially elevated in the Pliofilm cohort, but appeared to diminish with increasing duration of observation. Based on an evaluation of 1,165 white

male workers followed through 1981, Rinsky et al. (1987) found an SMR for multiple myeloma of 4.09 (95% CI 1.10–10.47), an increase that was statistically significant. Assessment of the cohort after an additional 6 and 15 years of follow-up yielded lower SMRs of 2.91 (95% CI 0.79–7.45) (Wong 1995) and 2.12 (95% CI 0.69–4.96) (Rinsky et al. 2002), respectively, risks that were no longer statistically significant. The risk of multiple myeloma did not increase with increasing level of cumulative exposure to benzene or with duration of employment, but this finding is particularly limited by the small number of deaths. No increases in mortality from multiple myeloma were found in other cohort mortality studies, including the NCI/CAPM study of Chinese workers (no observed deaths) (Yin et al. 1996a, 1996b), the Bloemen et al. (2004) study of chemical workers (SMR=0.72, 95% CI 0.15–2.10), and the Paci et al. (1989) study of shoe factory workers (no observed deaths in males; SMR=1.11 in females, 95% CI not calculated) summarized above. Additionally, mortality from multiple myeloma was not increased in a meta-analysis of 22 other cohorts of petroleum workers with potential exposure to benzene or benzene-containing petroleum products (Wong and Raabe 1997). The combined cohort consisted of 250,816 workers (from the United States, Canada, the United Kingdom, and Australia) who were observed over a period of 55 years from 1937 to 1991. The overall SMR for multiple myeloma was 0.93 (95% CI=0.81–1.07), and analyses by type of facility/industrial process (refinery, distribution, and crude oil production and pipeline workers) and duration of observation showed no differences in risk. The individual cohorts used in the meta-analysis also had no increased risk of multiple myeloma.

As summarized above, one of the early assessments of the Pliofilm cohort found an increased risk of mortality from multiple myeloma (Rinsky et al. 1987). The implications of this finding are unclear because the risk declined to non-significant levels in subsequent follow-ups (Rinsky et al. 2002; Wong 1995), and was not supported by the findings of other cohort mortality studies (Bloemen et al. 2004; Paci et al. 1989; Wong and Raabe 2000; Yin et al. 1996a, 1996b). Additionally, population-based and hospital-based case-control studies indicate that benzene exposure is not likely to be causally related to the risk of mulitple myeloma (Bezabeh et al. 1996; Heineman et al. 1992; Linet et al. 1987; Schnatter et al. 1996b; Sonoda et al. 2001; Wong and Raabe 1997). A meta-analysis of case-control studies found no significant association between occupational exposure to benzene and benzene-containing products and risk of multiple myeloma from sources categorized as benzene and/or organic solvents (summary odds ratio [OR]=0.74, 95% CI 0.60–0.90), petroleum (summary OR=1.11, 95% CI 0.96–1.28), or petroleum products (summary OR=1.08, 95% CI 0.89–1.33) (Sonoda et al. 2001).

Studies in animals provide supporting evidence for the carcinogenicity of inhaled benzene. As summarized below, inhalation exposure to benzene induced tumors at multiple sites in rats and mice, with a tendency towards induction of lymphomas in mice.

Carcinomas of the Zymbal gland and oral cavity were clearly increased in Sprague-Dawley rats that were exposed to 200–300 ppm benzene for 4–7 hours/day, 5 days/week for up to 104 weeks (Maltoni et al. 1982a, 1982b, 1983, 1985, 1989). The Zymbal gland is a specialized sebaceous gland and a site for benzene-induced tumors. Marginal increases in nasal cavity carcinomas, mammary tumors, and hepatomas were also observed. The significance of these findings is unclear because statistical analyses were not performed and there were no clearly increased incidences of Zymbal gland carcinoma or tumors at other sites in Sprague-Dawley rats that were similarly exposed to 100 or 300 ppm benzene for 6 hours/day, 5 days/week for life (Snyder et al. 1978b, 1984).

Benzene was carcinogenic in mice exposed to 100 or 300 ppm benzene for 6 hours/day, 5 days/week for 16 weeks and observed for 18 months or life (Cronkite 1986; Cronkite et al. 1984, 1985, 1989; Farris et al. 1993). These exposures consistently induced a variety of tumors, including leukemia (mainly thymic lymphoma) and Zymbal gland and ovarian tumors in C57BL/6 mice, and myelogenous leukemias, malignant lymphomas, and Zymbal gland and lung tumors in CBA/Ca mice. Incidences of lymphocytic lymphoma with thymic involvement were significantly increased in C57BL/6 mice similarly exposed to 300 ppm benzene for life (Snyder et al. 1980). Intermittent lifetime exposure to 300 ppm benzene (6 hours/day, 5 days/week on every third week) was more tumorigenic (in the Zymbal gland and lungs) to CD-1 and C57BL/6 mice than short-term exposure to 1,200 ppm (6 hours/day, 5 days/week for 10 weeks) followed by lifetime observation, although neither of these exposures induced leukemia (Snyder et al. 1988).

The cancer effect levels (CELs) for each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

EPA, IARC, and the Department of Health and Human Services have concluded that benzene is a human carcinogen. The Department of Health and Human Services (NTP 2005) determined that benzene is a known carcinogen based on human evidence showing a causal relationship between exposure to benzene and cancer. IARC (1987, 2004, 2007) classified benzene in Group 1 (carcinogenic to humans) based on sufficient evidence in both humans and animals. EPA (IRIS 2007) classified benzene in Category A (known human carcinogen) based on convincing evidence in humans supported by evidence from animal

studies. Under EPA's most recent guidelines for carcinogen risk assessment, benzene is characterized as a known human carcinogen for all routes of exposure based on convincing human evidence as well as supporting evidence from animal studies (IRIS 2007). Based on the Rinsky et al. (1981, 1987) human leukemia data, EPA derived a range of inhalation unit risk values of $2.2x10^{-6}$–$7.8x10^{-6}$ $(\mu g/m^3)^{-1}$ for benzene (IRIS 2007). For risks ranging from $1x10^{-4}$ to $1x10^{-7}$, the corresponding air concentrations for lifetime exposure range from 13.0–45.0 $\mu g/m^3$ (4–14 ppb) to 0.013–0.045 $\mu g/m^3$ (0.004–0.014 ppb), respectively. These risk levels are presented in Figure 3-1.

3.2.2 Oral Exposure

3.2.2.1 Death

Individual case reports of death from acute oral exposure to benzene have appeared in the literature since the early 1900s. The benzene concentrations encountered by the victims were often not known. However, lethal oral doses for humans have been estimated at 10 mL (8.8 g or 125 mg/kg for a 70-kg person) (Thienes and Haley 1972). Lethality in humans has been attributed to respiratory arrest, central nervous system depression, or cardiac collapse (Greenburg 1926). Accidental ingestion and/or attempted suicide with lethal oral doses of benzene have produced the following signs and symptoms: staggering gait; vomiting; shallow and rapid pulse; somnolence; and loss of consciousness, followed by delirium, pneumonitis, collapse, and then central nervous system depression, coma, and death (Thienes and Haley 1972). Ingestion of lethal doses may also result in visual disturbances and/or feelings of excitement and euphoria, which may quite suddenly change to weariness, fatigue, sleepiness, convulsion, coma, and death (Von Oettingen 1940).

Animal lethality data indicate that benzene is of low toxicity following acute oral exposure (O'Bryan and Ross 1988). Oral LD_{50} values for rats ranged from 930 to 5,600 mg/kg; the values varied with age and strain of the animals (Cornish and Ryan 1965; Wolf et al. 1956). Male Sprague-Dawley rats were given various doses of benzene to determine the LD_{50} (Cornish and Ryan 1965). The LD_{50} for nonfasted rats was found to be 930 mg/kg. In 24-hour fasted rats, the LD_{50} was 810 mg/kg. No increase in mortality was reported in Fischer 344 rats or $B6C3F_1$ mice treated with 600 mg/kg/day for up to 17 weeks (Huff et al. 1989; NTP 1986).

Sprague-Dawley rats (30–35 males, 30–35 females) were exposed to benzene by ingestion (stomach tube), in olive oil, at 0, 50, or 250 mg/kg/day for 4–5 days weekly for 52 weeks and then kept under supervision until spontaneous death (Maltoni et al. 1983). Exposure to 50 mg/kg/day benzene after

52 weeks resulted in deaths in 9 of 30 male (same as controls) and 2 of 30 female rats. At 250 mg/kg/day exposure, 13 of 35 males and 9 of 35 females died. For rats receiving only olive oil, 9 of 30 males and 0 of 30 females died. In a companion study, Sprague-Dawley rats were exposed to 500 mg/kg/day benzene by ingestion (stomach tube), in olive oil, 4–5 days/week for 92 weeks, and then kept under observation until spontaneous death (Maltoni et al. 1983). Mortality rates were the same as the controls.

In a chronic-duration oral study conducted by the NTP (1986), increased mortality was observed in male Fischer 344 rats exposed to 200 mg/kg/day benzene in corn oil, and in female Fischer 344 rats exposed to 50 mg/kg/day benzene. $B6C3F_1$ mice given 100 mg/kg/day also had increased mortality compared to control mice.

The LD_{50} values and all reliable LOAEL values for death in each species following acute and chronic exposure are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, musculoskeletal, hepatic, renal, endocrine, ocular, metabolic, or body weight effects in humans. Human and animal data pertaining to other systemic effects are presented below.

The highest NOAEL value and all reliable LOAEL values for systemic effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

Respiratory Effects. Male and female Fischer 344 rats and $B6C3F_1$ mice were given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days (NTP 1986). No histopathological lesions were observed in lungs, trachea, or mainstream bronchi. After chronic-duration exposure to 50, 100, or 200 mg/kg/day (male rats) or 25, 50, or 100 mg/kg/day (female rats, male and female mice), no histopathological lesions were observed in trachea, lungs, or mainstream bronchi in rats (NTP 1986). In mice, a significantly increased incidence of alveolar hyperplasia was observed at 50 and 100 mg/kg/day in females and at 100 mg/kg/day in males.

Cardiovascular Effects. No histopathological lesions were observed in cardiac tissue from male and female Fischer 344 rats or $B6C3F_1$ mice given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days (NTP 1986). After chronic-duration exposure to ≤200 mg/kg/day (male

Table 3-2 Levels of Significant Exposure to Benzene - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Human	once				126 (death)	Thienes and Haley 1972	
2	Rat (Sprague-Dawley)	once (G)				930 M (LD50)	Cornish and Ryan 1965	
3	Rat (Wistar)	once (GO)				5600 M (LD50)	Wolf et al. 1956	
Systemic								
4	Rat (Sprague-Dawley)	Gd 6-15 daily (G)	Renal	1000 F			Exxon 1986	
			Dermal		50 F (alopecia of hindlimbs and trunk)			
			Bd Wt	500 F	1000 F (body weight decreased 11%)			
			Other	50 F	250 F (decreased food consumption)			
5	Rat (CF)	1-3 d	Hepatic		1402 M (increased liver weight, biochemical changes)		Pawar and Mungikar 1975	
Neurological								
6	Human	once				126 (muscular incoordination, unconsciousness)	Thienes and Haley 1972	

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
7	Rat (Sprague-Dawley)	1 d			88 M (slight CNS depression)	1870 M (tremors)	Cornish and Ryan 1965	
8	Rat (Sprague-Dawley)	once (G)			950 M (altered neurotransmitter concentrations)		Kanada et al. 1994	
Reproductive								
9	Rat (Sprague-Dawley)	Gd 6-15 daily (G)		1000 F			Exxon 1986	
Developmental								
10	Rat (Sprague-Dawley)	Gd 6-15 daily (G)		1000 F			Exxon 1986	
11	Mouse (ICR/SIM)	Gd 8-12 (GO)			1300 F (decreased pup weight on neonatal days 1-3)		Seidenberg et al. 1986	
INTERMEDIATE EXPOSURE								
Death								
12	Rat (Sprague-Dawley)	52 wk 4-5 d/wk 1 x/d (GO)				250 F (9/35 died)	Maltoni et al. 1983, 1985	

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Systemic								
13	Rat (Sprague-Dawley)	52 wk 4-5 d/wk 1 x/d (GO)	Bd Wt	50	250 (body weight decreased 19%)		Maltoni et al. 1983, 1985	
14	Rat (F-344/N)	<1 yr 5 d/wk (GO)	Hemato		50 M (lymphocytopenia and leukocytopenia)		NTP 1986	
					25[b] F (lymphocytopenia and leukocytopenia)			
			Bd Wt	100	200 M (body weight decreased 11% or more in 25 weeks)			

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
15	Rat (F-344/N)	60-120 d 5 d/wk (GO)	Resp	600			NTP 1986	
			Cardio	600				
			Gastro	600				
			Hemato		200 (dose-related leukopenia at 60 days)			
					25[b] (dose-related leukopenia at 120 days)			
			Musc/skel	600				
			Hepatic	600				
			Renal	600				
			Endocr	600				
			Bd Wt		200 (body weight decreased 14% in males and 16% in females)			
16	Rat (Fischer- 344)	6 wk 5 d/wk (GO)	Hepatic	400 M			Taningher et al. 1995	
			Bd Wt	400 M				
17	Rat (Wistar)	6 mo 5 d/wk (GO)	Hemato	1 F		50 F (leukopenia, erythrocytopenia)	Wolf et al. 1956	

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
18	Mouse (C57BL/6)	28 d (W)	Bd Wt	1000 M			Fan 1992	
19	Mouse (CD-1)	4 wk ad lib (W)	Hemato			8 M (erythrocytopenia, increased mean corpuscular volume; leukopenia)	Hsieh et al. 1988b	
20	Mouse (CD-1)	4 wk (W)	Hemato			31.5 M (decreased erythrocyte, leukocyte counts)	Hsieh et al. 1990	
			Hepatic	31.5 M				
			Renal	31.5 M				
			Bd Wt	31.5 M				
21	Mouse (B6C3F1)	<1 yr 5 d/wk (GO)	Hemato		25 (lymphocytopenia)		NTP 1986	
			Bd Wt	100				

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
22	Mouse (B6C3F1)	60-120 d 5 d/wk (GO)	Resp	600			NTP 1986	
			Cardio	600				
			Gastro	600				
			Hemato	200 F	50 M[b] (dose-related leukopenia)			
				25	400 F (dose-related leukopenia)			
			Musc/skel	600				
			Hepatic	600				
			Renal	600				
			Endocr	600				
			Bd Wt	600				
23	Mouse (B6C3F1)	30 d ad lib (W)	Hemato	12 F	195 F (decreased leukocytes)	350 F (decreased hemoglobin, hematocrit, leukocytes, MCV, and MCH)	Shell 1992	
			Hepatic	350 F				
			Renal	350 F				
			Bd Wt	350 F				
			Other		12 F (decreased fluid intake)			

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Immuno/ Lymphoret								
24	Rat (F-344/N)	60-120 d 5 d/wk (GO)			200 (lymphopenia at 60 days, lymphoid depletion in B-cell of the spleen)		Huff et al. 1989; NTP 1986	
					25 F dose-related lymphopenia at 120 days)			
25	Rat (Wistar)	6 mo 5 d/wk (GO)		1 F	50 F (leukopenia)		Wolf et al. 1956	
26	Mouse (C57BL/6)	28 d (W)			27 M (decreased number of splenocytes and IL-2 production)		Fan 1992	
27	Mouse (CD-1)	4 wk ad lib (W)				8 M (leukopenia and lymphopenia; enhanced splenic lymphocyte proliferation)	Hsieh et al. 1988b	
28	Mouse (CD-1)	4 wk (W)				31.5 M (reduction in thymus mass; suppresion of both B- and T-cell mitogeneses; suppressed IL-2 secretions; leukopenia, lymphopenia)	Hsieh et al. 1990	

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
29	Mouse (CD-1)	4 wk ad lib (W)				40 M (elevated corticosterone levels; T-lymphocyte suppression)	Hsieh et al. 1991	
30	Mouse (B6C3F1)	60-120 d 5 d/wk (GO)		25 M[b] 200 F	50 M[b] (dose-related lymphopenia) 400 F		Huff et al. 1989; NTP 1986	
31	Mouse (B6C3F1)	30 d ad lib (W)			12 F (decreased leukocytes)		Shell 1992	
Neurological								
32	Rat (F-344/N)	60-120 d 5 d/wk (GO)		600			NTP 1986	
33	Mouse (CD-1)	4 wk ad lib (W)			8 M (fluctuation of neurotransmitter levels)		Hsieh et al. 1988a	
34	Mouse (CD-1)	4 wk (W)			31.5 M (decreased NE, DA, 5-HT)		Hsieh et al. 1990	
35	Mouse (CD-1)	4 wk ad lib (W)		8 M	40 M (increased hypothalmic NE and VMA)		Hsieh et al. 1991	

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
36	Mouse (B6C3F1)	60-120 d 5 d/wk (GO)		200	400 (intermittent tremors)		NTP 1986	
37	Mouse (B6C3F1)	30 d ad lib (W)		195 F	350 F (decreased brain weight)		Shell 1992	
Reproductive								
38	Rat (F344/N)	17 wk 5 d/wk (GO)		600			NTP 1986	
39	Mouse (B6C3F1)	17 wk 5 d/wk (GO)		600			NTP 1986	
Cancer								
40	Rat (Sprague-Dawley)	52 wk 4-5 d/wk 1 x/d (GO)				50 F (CEL: Zymbal gland carcinoma in 2/30; oral cavity carcinoma)	Maltoni et al. 1983, 1985	
41	Rat (Sprague-Dawley)	52 wk 4-5 d/wk 1 x/d (GO)				50 (CEL: Zymbal gland carcinoma)	Maltoni et al. 1989	
						250 (CEL: nasal cavity carcinoma, fore-stomach liver angiosarcoma; Zymbal gland carcinoma)		

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
42	Mouse (RF/J)	52 wk 4-5 d/wk (GO)				500 (CEL: mammary pulmonary leukemias)	Maltoni et al. 1989	
CHRONIC EXPOSURE								
Death								
43	Rat (F-344/N)	2 yr 5 d/wk (GO)				200 M (30/50 died) 50[b] F (14/50 died)	NTP 1986	
44	Mouse (B6C3F1)	2 yr 5 d/wk (GO)				100 (41/50 males died, 35/50 females died)	NTP 1986	
Systemic								
45	Human	6.1 yr (avg) (Occup)	Hemato		0.29[c] (reduced WBC and platelet counts, approximately 7-18% lower than control values)		Lan et al. 2004a	Route-to-route extrapolation from the reported LOAEL of 0.57 ppm for occupational exposure was used by ATSDR to estimate equivalent oral dose.

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
46	Rat (F-344/N)	2 yr 5 d/wk (GO)	Resp	200 M 100[b] F			Huff et al. 1989; NTP 1986	
			Cardio	200 M 100[b] F				
			Gastro	100	200 M (hyperkeratosis and acanthosis in nonglandular forestomach)			
			Hemato		50 M (lymphocytopenia and leukocytopenia) 25[b] F (lymphocytopenia and leukocytopenia)			
			Musc/skel	200 M 100[b] F				
			Hepatic	200 M 100[b] F				
			Renal	200 M 100[b] F				
			Endocr	200 M 100[b] F				
			Dermal	200 M 100[b] F				
			Ocular	200 M 100[b] F				

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
47	Rat (F-344/N)	2 yr 5 d/wk (GO)	Bd Wt	100 M		200 M (body weights decreased 23% in 103 weeks)	Huff et al. 1989; NTP 1986	
48	Rat (Sprague-Dawley)	92 wk 4-5 d/wk 1 x/d (GO)	Hemato			500 (decreased RBCs and WBCs after 84 weeks)	Maltoni et al. 1983, 1985	
			Bd Wt		500 (decreased body weights in 92 weeks)			

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
49	Mouse (B6C3F1)	2 yr 5 d/wk (GO)	Resp	50 M 25 F[b]	100 M 50 F[b] (alveolar hyperplasia)		NTP 1986	
			Cardio	100				
			Gastro		25 (epithelial hyperplasia and hyperkeratosis of forestomach)			
			Hemato		25 (lymphocytopenia; increased frequency of micronucleated normochromatic peripheral erythrocytes)			
			Musc/skel	100				
			Hepatic	100				
			Renal	100				
			Endocr		25 (hyperplasia of adrenal gland and harderian gland)			
			Dermal	100				
			Bd Wt	50	100 (mean body weight decreased 10% in 47 weeks to 19% in 103 weeks in males; decreased 14-15% in week 99-103 in females)			

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Immuno/ Lymphoret								
50	Rat (F-344/N)	2 yr 5 d/wk (GO)			50 M (lymphoid depletion of spleen and thymus)		Huff et al. 1989; NTP 1986	
					25[b] F (lymphoid depletion of spleen and thymus)			
51	Rat (Sprague-Dawley)	92 wk 4-5 d/wk 1 x/d (GO)			500 (decreased WBCs after 84 weeks)		Maltoni et al. 1983, 1985	
52	Mouse (B6C3F1)	2 yr 5 d/wk (GO)			25 (lymphopenia, hematopoietic hyperplasia in the bone marrow, splenic hematopoiesis)		Huff et al. 1989; NTP 1986	
Neurological								
53	Rat (F-344/N)	2 yr 5 d/wk (GO)		200 M 100[b] F			NTP 1986	
54	Mouse (B6C3F1)	2 yr 5 d/wk (GO)		100			NTP 1986	

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Reproductive								
55	Rat (F-344/N)	2 yr 5 d/wk (GO)		200 M 50[b] F		100 F (endometrial polyps)	NTP 1986	
56	Mouse (B6C3F1)	2 yr 5 d/wk (GO)			25 (preputial gland hyperplasia in males; ovarian hyperplasia and senile atrophy in females)		NTP 1986	
Cancer								
57	Rat (F-344/N)	2 yr 5 d/wk (GO)				50 M (CEL: squamous cell papillomas and carcinomas of the oral cavity)	Huff et al. 1989; NTP 1986	
						25[b] F (CEL: Zymbal gland carcinomas)		
58	Rat (Sprague-Dawley)	92 wk 4-5 d/wk 1 x/d (GO)				500 (CEL: Zymbal gland carcinoma; oral and nasal cavity carcinoma; angiosarcoma of the liver)	Maltoni et al. 1983, 1985	

Table 3-2 Levels of Significant Exposure to Benzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
59	Rat (Wistar)	104 wk 4-5 d/wk 1 x/d (GO)				500 (CEL: Zymbal gland and oral and nasal cavity carcinoma; angiosarcoma of the liver)	Maltoni et al. 1989	
60	Rat (Sprague-Dawley)	104 wk 5 d/wk (GO)				50 M (CEL: Zymbal gland carcinoma)	Maltoni et al. 1989	
61	Mouse (B6C3F1)	2 yr 5 d/wk (GO)				25 (CEL: harderian gland adenoma, lymphoma in males; CEL: lymphoma in females)	Huff et al. 1989; NTP 1986	
62	Mouse (Swiss)	78 wk 4-5 d/wk 1 x/d (GO)				500 (CEL: mammary tumors, lung tumors, Zymbal gland)	Maltoni et al. 1989	

a The number corresponds to entries in Figure 3-2.

b Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 3-2. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

c Study results used to derive a chronic-duration oral minimal risk level (MRL) of 0.0005 mg/kg/day based on route-to-route extrapolation, as described in detail in Chapter 2 and Appendix A. Benchmark dose (BMD) analysis was performed on B-lymphocyte counts to select a point of departure, which was adjusted for intermittent exposure. An equivalent oral dose was estimated based on route-to-route extrapolation to determine a point of departure for deriving a chronic-duration oral MRL for benzene, which was divided by an uncertainty factor of 30 (10 for human variability and 3 for uncertainty in route-to-route extrapolation).

ad lib = ad libitum; Bd Wt = body weight; CEL = cancer effect level; CNS = central nervous system; d = day(s); DA = dopamine; F = female; (F) = feed; (G) = gavage; Gd = gestational day; (GO) = gavage in oil; Hemato = hematological; 5-HT = 5-hydroxystryptamine; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; mo = month(s); NE = norepinephrine; NOAEL = no-observed-adverse-effect level; NS = not specified; RBC = red blood cells; VMA = vanillin mandelic acid; (W) = water; WBC = white blood cells; wk = week(s); yr = year(s); x = times

Figure 3-2 Levels of Significant Exposure to Benzene - Oral

Acute (≤14 days)

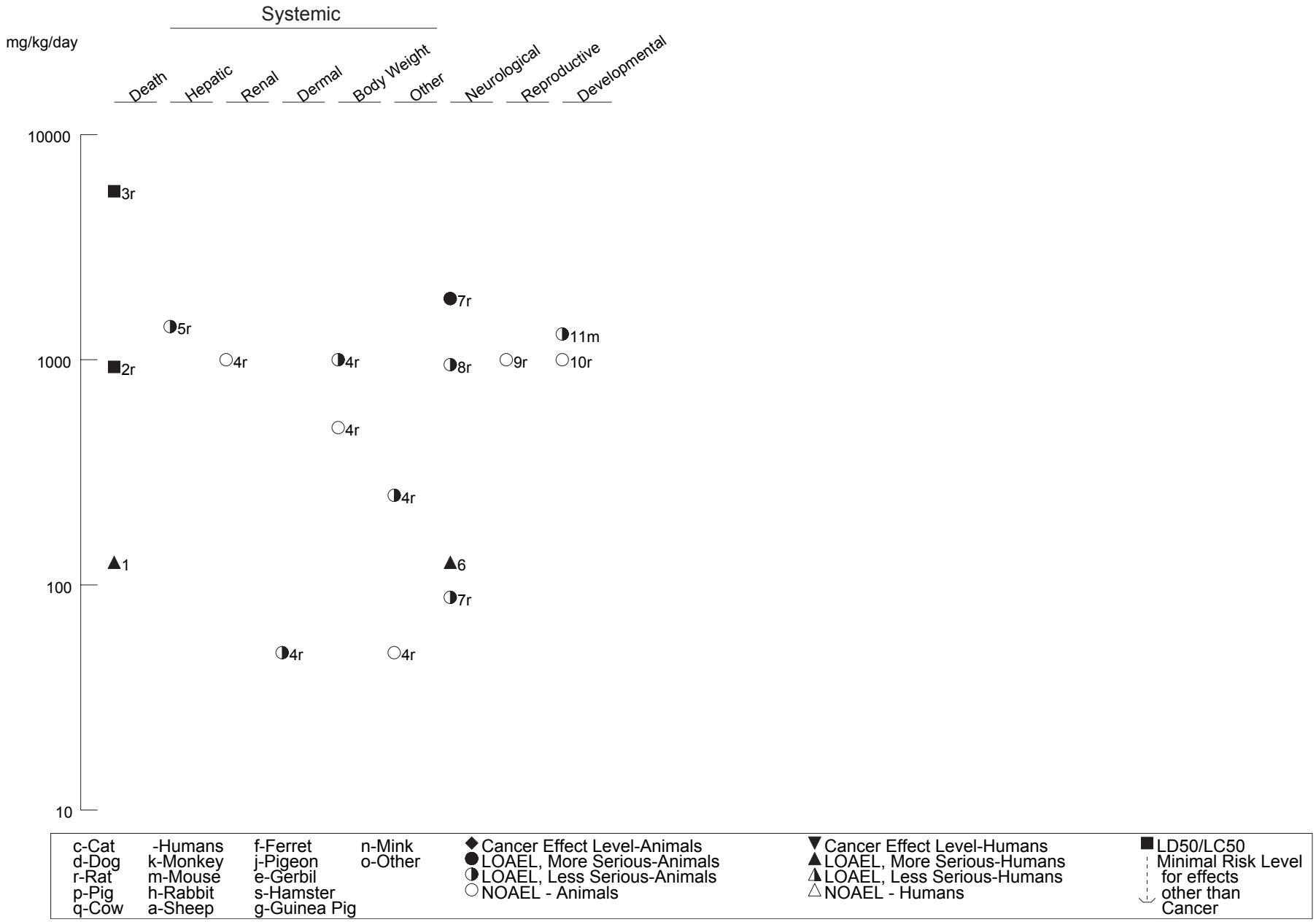
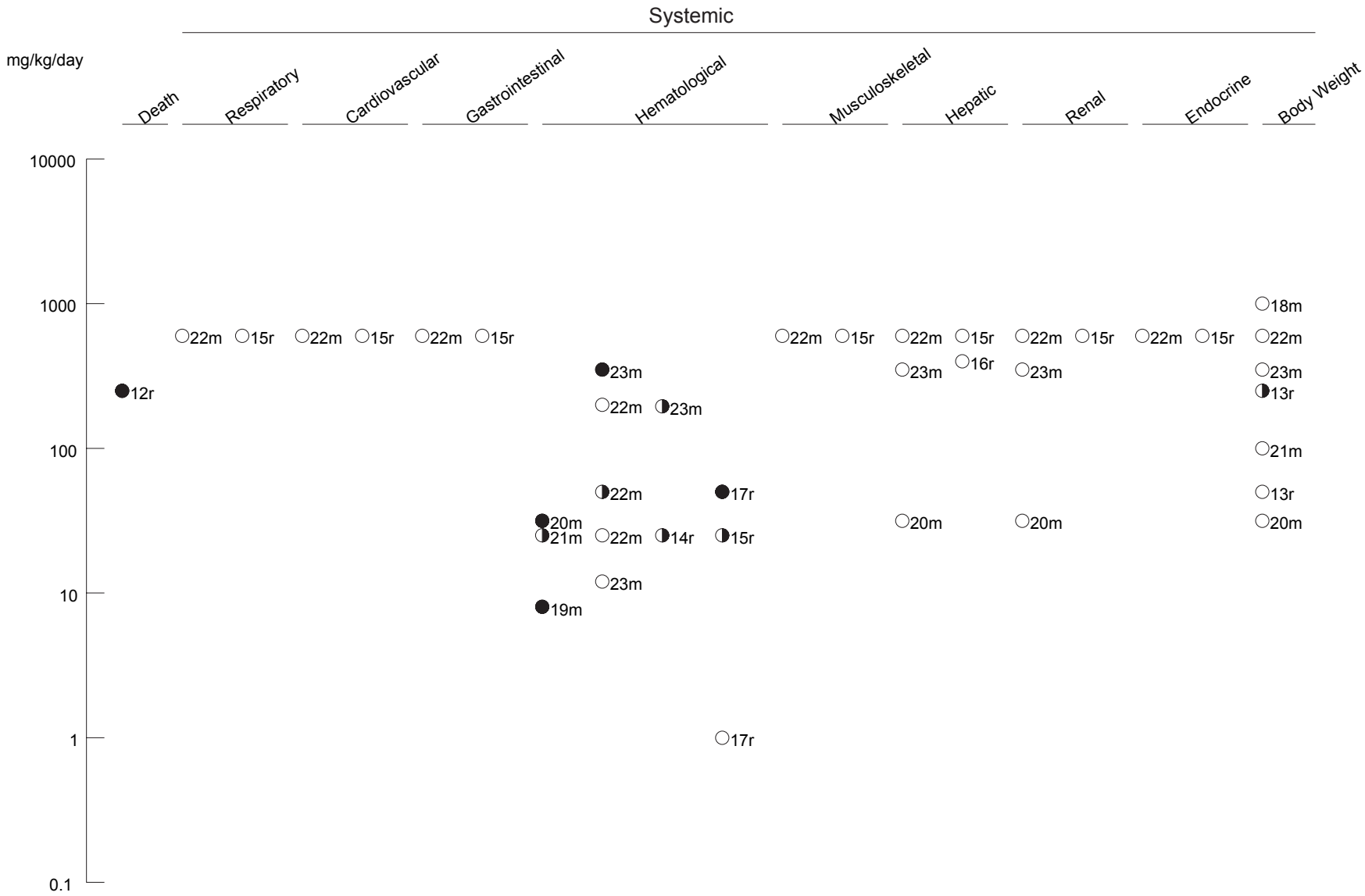


Figure 3-2 Levels of Significant Exposure to Benzene - Oral *(Continued)*

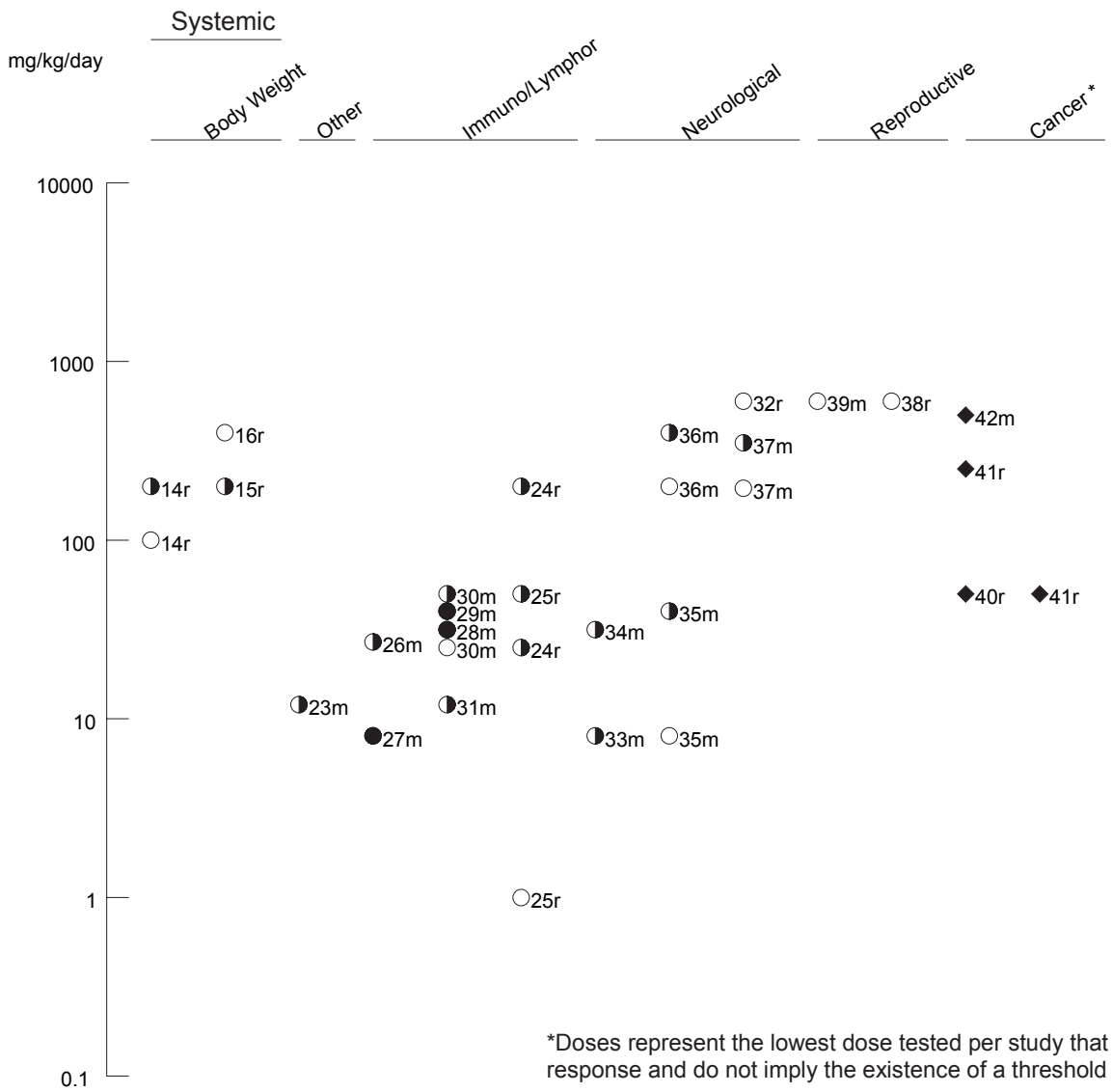
Intermediate (15-364 days)



c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level for effects other than Cancer
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◭ LOAEL, Less Serious-Humans	
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	
q-Cow	a-Sheep	g-Guinea Pig				

Figure 3-2 Levels of Significant Exposure to Benzene - Oral *(Continued)*

Intermediate (15-364 days)

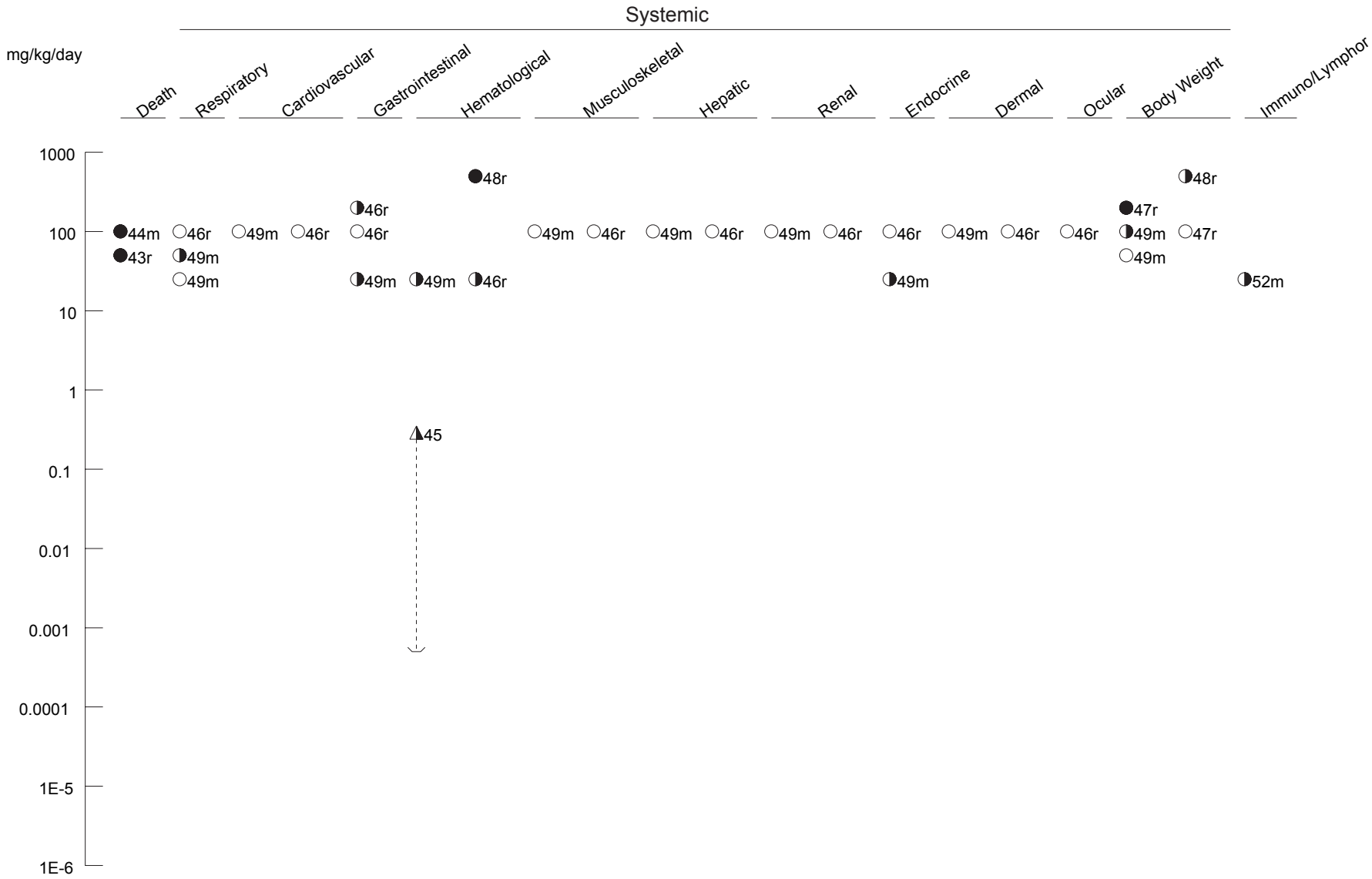


*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint.

c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level for effects other than Cancer
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◭ LOAEL, Less Serious-Humans	
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	
q-Cow	a-Sheep	g-Guinea Pig				

Figure 3-2 Levels of Significant Exposure to Benzene - Oral *(Continued)*

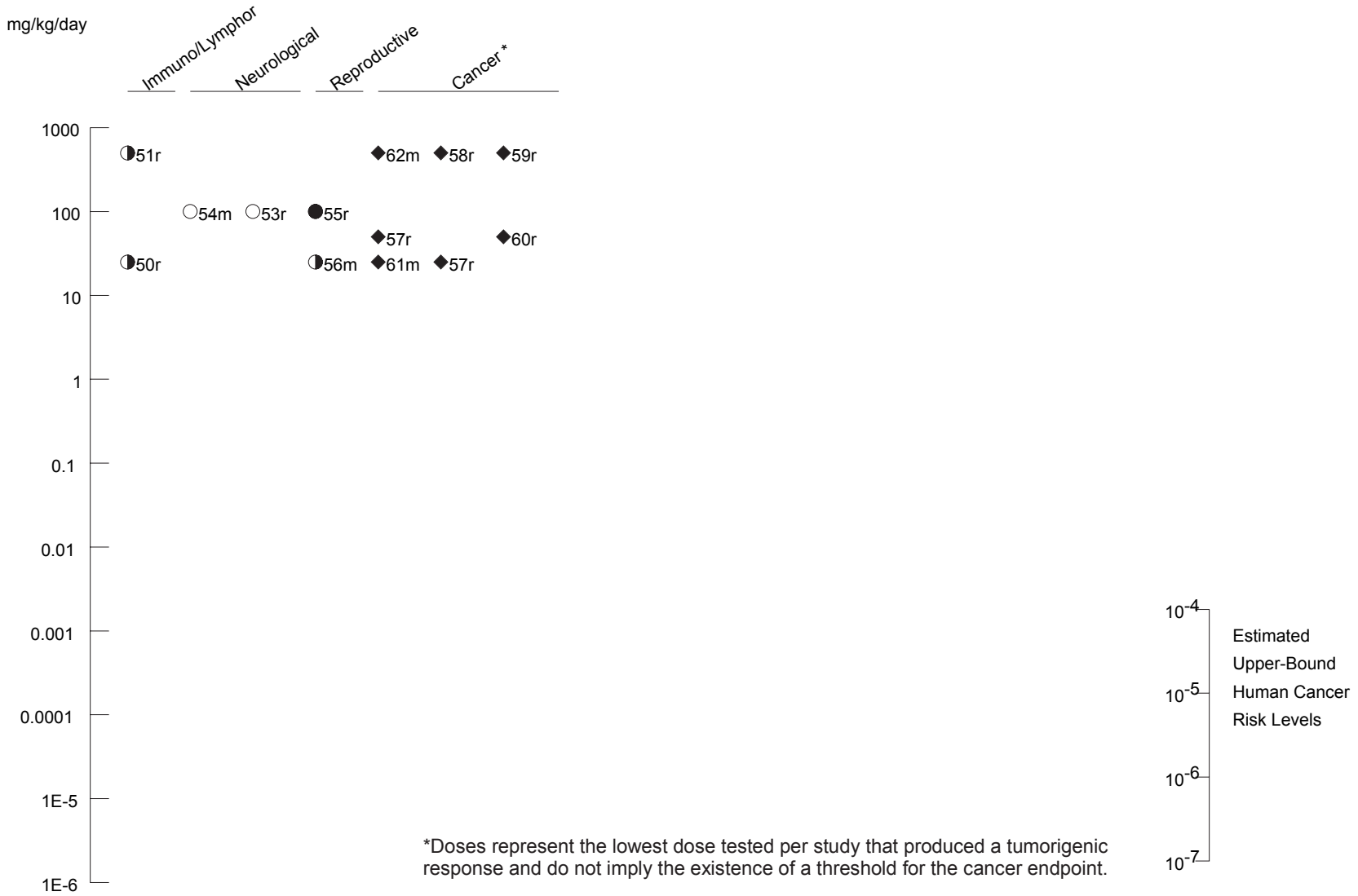
Chronic (≥365 days)



c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level for effects other than Cancer
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◭ LOAEL, Less Serious-Humans	
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	
q-Cow	a-Sheep	g-Guinea Pig				

Figure 3-2 Levels of Significant Exposure to Benzene - Oral *(Continued)*

Chronic (≥365 days)



*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint.

c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level for effects other than Cancer
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◭ LOAEL, Less Serious-Humans	
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	
q-Cow	a-Sheep	g-Guinea Pig				

rats) or ≤100 mg/kg/day (female rats, male and female mice) no histopathological lesions were observed in the heart (NTP 1986).

Gastrointestinal Effects. A man swallowed an unspecified amount of benzene and survived, but developed an intense toxic gastritis and later pyloric stenosis (Greenburg 1926).

No histopathological lesions were observed in esophageal and stomach tissue or in the small intestine and colon from male and female Fischer 344 rats or $B6C3F_1$ mice given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days (NTP 1986). After chronic-duration exposure to 50–200 mg/kg/day (male rats) or 25–100 mg/kg/day (female rats, male and female mice), male rats exhibited hyperkeratosis and acanthosis in the nonglandular forestomach at 200 mg/kg/day, and mice exhibited epithelial hyperplasia and hyperkeratosis in the forestomach at 25 mg/kg/day (NTP 1986).

Hematological Effects. Prior to 1913, benzene was used as a treatment for leukemia. Benzene was given in gelatin capsules starting with 43 mg/kg/day and increasing to 71 mg/kg/day for unspecified durations (Selling 1916). Leukemia patients showed a great reduction in leukocyte count and multiple hemorrhages with advanced anemia. However, it is difficult to determine which effects were due to the leukemia and which were due to the benzene treatment.

Intermediate-duration studies in animals have revealed decreases in numbers of erythrocytes and leukocytes following exposure to benzene. Male and female Fischer 344 rats and $B6C3F_1$ mice were given oral doses of 0, 25, 50, 100, 200, 400, and 600 mg/kg/day benzene in corn oil for 120 days. Dose-related leukopenia and lymphopenia were observed at 200 and 600 mg/kg/day for both male and female rats killed on day 60, and at all doses in female rats killed on day 120. Dose-related leukopenia and lymphopenia were observed in male mice at 50 mg/kg/day and female mice at 400 mg/kg/day for 120 days, but not for 60 days. Mice exposed to 8 mg/kg/day in the drinking water for 4 weeks had decreased numbers of erythrocytes, increased mean corpuscular volumes, and decreased numbers of lymphocytes (Hsieh et al. 1988b, 1990). Female $B6C3F_1$ mice were exposed to 0, 12, 195, or 350 mg/kg/day benzene in drinking water for 30 days (Shell 1992). Decreased hemoglobin, hematocrit, leukocytes, MCV, and MCH were observed at 350 mg/kg/day. Decreased leukocytes also occurred at 195 mg/kg/day. Decreased leukocytes also occurred at 195 mg/kg/day. Rats gavaged with 50 mg/kg/day of benzene 5 days/week for 6 months also had decreased numbers of erythrocytes and leukocytes (Wolf et al. 1956). One chronic-duration study showed that gavage doses of 25 mg/kg/day resulted in leukopenia and/or lymphocytopenia in both rats and mice, both at the interim sacrifices at 3–18 months, and at the

end of 2 years (Huff et al. 1989; NTP 1986). Increased frequency of micronucleated normochromatic peripheral erythrocytes was observed in mice at 25 mg/kg/day after 2 years. Sprague-Dawley rats were exposed to 500 mg/kg benzene by ingestion (stomach tube), in olive oil, once daily, 4–5 days/week for 92 weeks, and then kept under observation until spontaneous death (Maltoni et al. 1983). Decreased erythrocytes and leukocytes were observed after 84 weeks.

Musculoskeletal Effects. No histopathological lesions were observed in femoral tissue from male and female Fischer 344 rats or B6C3F$_1$ mice given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days, or in the sternebrae, femur, or vertebrae from Fischer 344 rats and mice exposed to 50–200 mg/kg/day (male rats) or 25–100 mg/kg/day (female rats, male and female mice) for 2 years (NTP 1986).

Hepatic Effects. Acute oral administration of 1,402 mg/kg/day benzene for 3 days induced hepatic changes in rats evidenced by increased liver weight, decreased protein in the postmitochondrial supernatant fractions (9,000 times specific gravity), and changes in hepatic drug metabolism and lipid peroxidation (Pawar and Mungikar 1975). The initiation-promotion-progression (IPP) model for the induction of malignant neoplasms in the liver was evaluated for benzene in male and female Sprague-Dawley rats (Dragan et al. 1993). Initiation was begun in 5-day-old rats with administration of a single intraperitoneal injection of diethylnitrosamine during the time when the liver is undergoing rapid growth. Promotion began at 6 months of age with phenobarbital in the feed, and continued into young adulthood. Partial hepatectomy was performed, and at the height of the regenerative proliferation phase following the hepatectomy, benzene (1 g/kg) was administered by gavage; phenobarbital treatment was maintained after the administration of benzene. A slight increase in the incidence of altered hepatic foci was observed after initiation and promotion. Few hepatic foci were observed in the livers of male Fischer 344 rats treated by gavage with 400 mg/kg/day benzene in corn oil for 5 days/week for 6 weeks (Taningher et al. 1995). No histopathological non-neoplastic lesions were observed in hepatic tissue from male and female Fischer 344 rats given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days or in male rats exposed to 50–200 mg/kg/day and female rats exposed to 25–100 mg/kg/day for 2 years (NTP 1986).

Female B6C3F$_1$ mice were exposed to 0, 12, 195, or 350 mg/kg/day benzene in drinking water for 30 days (Shell 1992). No adverse liver effects, as evidenced by gross necropsy, liver weight determination, and serum levels of hepatic enzymes, were observed. Oral administration of 31.5 mg/kg/day benzene continuously in drinking water for 4 weeks did not affect liver weight in CD-1

mice (Hsieh et al. 1990). No histopathological non-neoplastic lesions effects were observed in hepatic tissue from male and female B6C3F$_1$ mice given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days, or in male and female mice exposed to 25–100 mg/kg/day for 2 years (NTP 1986).

Renal Effects. Female Sprague-Dawley rats were dosed by gavage with 0, 50, 250, 500, or 1,000 mg/kg/day benzene on Gd 6–15 and killed on Gd 20 (Exxon 1986). No adverse effects were noted in the kidneys based on gross necropsy. No adverse effects based on histological examination were observed on renal tissue or the urinary bladder from male and female Fischer 344 rats given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days or in male rats exposed to 50–200 mg/kg/day and female rats exposed to 25–100 mg/kg/day for 2 years (NTP 1986). Female B6C3F$_1$ mice were exposed to 0, 12, 195, or 350 mg/kg benzene in drinking water for 30 days (Shell 1992). No adverse effects were observed in the kidneys, based on kidney weights, gross examination, and blood urea nitrogen and creatinine determinations. Oral administration of 31.5 mg/kg/day benzene continuously in drinking water for 4 weeks did not affect kidney weight in CD-1 mice (Hsieh et al. 1990). No adverse effect based on histological examination was observed on renal tissue or the urinary bladder from male and female B6C3F$_1$ mice given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days, or in male and female mice exposed to 25–100 mg/kg/day for 2 years (NTP 1986).

Endocrine Effects. No histopathological lesions were observed in salivary, thyroid, parathyroid, pancreas, adrenal, or pituitary glands from male and female Fischer 344 rats or B6C3F$_1$ mice given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days (NTP 1986). In the companion chronic-duration oral study, male Fischer 344 rats were exposed to 25, 50, 100, or 200 mg/kg/day benzene, while female rats received 25, 50, or 100 mg/kg/day benzene. Hyperplasia of the Zymbal gland was increased in low-dose males and in mid-dose females. In the adrenal gland, hyperplasia was observed in both sexes (males: 27 and 4% at 50 and 200 mg/kg, respectively; females: 34% at 25 mg/kg). In the thyroid gland, incidences of C-cell hyperplasia were 14, 26, 15, and 15% in males treated with 0, 50, 100, and 200 mg/kg, respectively. Analysis of the pituitary gland showed incidence of hyperplasia in males treated with 0, 50, 100, and 200 mg/kg at 6, 16, 20, and 10%, respectively; in females treated with 0, 25, 50, and 100 mg/kg at 11, 20, 10, and 14%, respectively. None of the increased incidences of hyperplasia in these glands were considered to be treatment-related by NTP. The non-dose-related increase of hyperplasia of the Zymbal gland could represent a progression to the neoplasms (see Section 3.2.2.7). In mice, Zymbal gland lesions showed epithelial hyperplasia in

males (0, 9, 30, and 26%) and in females (2, 3, 5, and 19%) exposed to 0, 25, 50, or 100 mg/kg, respectively. Hyperplasia of the adrenal cortex occurred at incidences of 4, 67, 29, and 9% in males and 10, 43, 68, and 13% in females, respectively. Hyperplasia of the harderian gland occurred at incidences of 0, 11, 22, and 15% in males and 13, 23, 22, and 21% in females, respectively (NTP 1986).

Dermal Effects. A case of accidental poisoning in which the patient survived but developed an odd skin condition consisting of swelling and edema has been reported (Greenburg 1926).

Female Sprague-Dawley rats were dosed by gavage with 0, 50, 250, 500, or 1,000 mg/kg/day benzene daily on Gd 6–15 and killed on Gd 20 (Exxon 1986). Alopecia of the hind limbs and trunk was noted in all dose groups.

No histopathological lesions were observed in the skin of male and female Fischer 344 rats and B6C3F$_1$ mice after chronic oral exposure to 50–200 mg/kg/day (male rats) or 25–100 mg/kg/day (female rats and male and female mice) (NTP 1986).

Ocular Effects. No histopathological lesions were noted in the eyes of male and female Fischer 344 rats and B6C3F$_1$ mice after chronic-duration oral exposure to 50–200 mg/kg/day (male rats) or 25–100 mg/kg/day (female rats and male and female mice) (NTP 1986).

Body Weight Effects. No significant change in body weight was observed in male Fischer 344 rats treated by gavage with 400 mg/kg/day benzene in corn oil for 5 days/week for 6 weeks (Taningher et al. 1995). Body weight was unaffected in male and female Fischer 344 rats given oral doses of 0, 25, 50, or 100 mg/kg/day benzene in corn oil for 120 days (NTP 1986). However, animals receiving 200, 400, or 600 mg/kg/day benzene exhibited a 14–22% decrease in body weight after 120 days. Female Sprague-Dawley rats were dosed by gavage with 0, 50, 250, 500, or 1,000 mg/kg benzene daily on Gd 6–15 and killed on Gd 20 (Exxon 1986). Maternal body weight decreased 11% at the high dose. C57BL/6 male mice were given benzene at concentration levels of 200 and 1,000 mg/L (assumed benzene intake of 27 and 154 mg/kg/day) in drinking water for 28 days (Fan 1992). Control groups were given untreated tap water. Groups of mice were killed on day 7, 14, 21, and 28 of administration, and on day 7, 14, 21, and 28 after the last administration of benzene at 200 mg/L. There was no effect of treatment on body weight. Female B6C3F$_1$ mice were exposed to 0, 12, 195, or 350 mg/kg/day benzene in drinking water for 30 days (Shell 1992). There was no significant effect on body weight at the highest treatment level. Oral administration of 31.5 mg/kg/day benzene continuously in drinking water for 4 weeks did not affect

body weight in CD-1 mice (Hsieh et al. 1990). No adverse effect was observed on body weight of male and female $B6C3F_1$ mice given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days (NTP 1986).

Sprague-Dawley rats were exposed to benzene by gavage in olive oil, at 0, 50, or 250 mg/kg/day body weight for 4–5 days/week for 52 weeks, and then kept under supervision until spontaneous death (Maltoni et al. 1983, 1985). A 19% decrease in body weight was reported in animals exposed to 250 mg/kg benzene for 52 weeks. In a companion study, Sprague-Dawley rats were exposed to 500 mg/kg benzene by ingestion (stomach tube), in olive oil, once daily, 4–5 days/week for 92 weeks, and then kept under observation until spontaneous death (Maltoni et al. 1983). Decreased body weight was observed after 92 weeks. In a chronic-duration oral study, male Fischer 344 rats exhibited a decrease in body weight of 11% or more after 25 weeks exposure to doses of 200 mg/kg/day benzene in corn oil (NTP 1986). Females rats and male and female $B6C3F_1$ mice in the same study exposed to doses up to 100 mg/kg/day benzene did not show any change in body weight after 12 months of exposure, or after 2 years exposure (female rats). Male and female mice exhibited body weight effects after chronic exposure. In male mice given 100 mg/kg, mean body weights decreased from 10% after 47 weeks to 19% in 103 weeks of exposure relative to controls. In female mice given 100 mg/kg, mean body weights decreased 14–15% in weeks 99–103 of exposure (NTP 1986).

Other Systemic Effects. C57BL/6 male mice were given benzene at concentration levels of 200 and 1,000 mg/L (assumed benzene intake of 27 and 154 mg/kg/day) in drinking water for 28 days (Fan 1992). Control groups were given untreated tap water. Groups of mice were killed on day 7, 14, 21, and 28 of administration, and on day 7, 14, 21, and 28 after the last administration of benzene at 200 mg/L. There was no effect of treatment on food or water consumption. Female Sprague-Dawley rats were dosed by gavage with 0, 50, 250, 500, or 1,000 mg/kg/day on Gd 6–15 and killed on Gd 20 (Exxon 1986). Decreased feed consumption was noted at doses of 250 mg/kg and above, and body weight decreased 11% at the high dose. Female $B6C3F_1$ mice were exposed to 0, 12, 195, or 350 mg/kg/day benzene in drinking water for 30 days (Shell 1992). Decreased fluid consumption was observed at >12 mg/kg.

3.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans after oral exposure to benzene.

Oral administration of benzene to CD-1 mice produced an immunotoxic effect on both the humoral and cellular immune responses (Hsieh et al. 1988b). Exposure to benzene at 8, 40, or 180 mg/kg/day for 4 weeks caused a significant dose-response reduction of total peripheral blood leukocytes and erythrocytes. Lymphocytes, but not neutrophils or other leukocytes, were decreased in number. Splenic lymphocyte proliferative response to B- and T-cell mitogens was biphasic—enhanced in the 8 mg/kg/day dosage group and depressed in the 40 and 180 mg/kg/day dosage groups. Cell-mediated immunity evaluated in mixed-lymphocyte reaction and in the ^{51}Cr-release assay showed a similar biphasic response. Antibody production was significantly suppressed in mice dosed at 40 and 180 mg/kg/day. The results indicate that administration of 40 mg/kg/day benzene has an immunosuppressive effect evident in decreased immune functions evaluated in *in vitro* assays for cell-mediated immunity and antibody production. A dose-related decrease in spleen weight was observed, which was significant only in the 180 mg/kg/day group.

C57BL/6 male mice were given benzene at concentration levels of 200 and 1,000 mg/L (assumed benzene intake of 27 and 154 mg/kg/day) in drinking water for 28 days (Fan 1992). Control groups were given untreated tap water. Mice were sacrificed on days 7, 14, 21, and 28 of administration. Selected mice of the 27 mg/kg/day dose group were sacrificed on postexposure days 7, 14, and 21 in order to assess the postexposure time course of the benzene toxicity. At 27 mg/kg/day, a decreased number of splenocytes was observed on day 21 and 28 of exposure. At 154 mg/kg/day, spleen cell numbers decreased significantly as a function of time in mice treated for 14, 21, and 28 days. Benzene treatment for 3 weeks raised natural killer (NK) cell activity significantly at both doses. However, after another week of benzene treatment, NK cell activity resumed to control levels. Significant depression of interleukin-2-(IL-2) production was detected in both levels for 28 days. The NK cell activity showed normal levels on day 7, 14, and 21 after the last administration of benzene exposure for 28 days at 27 mg/kg/day. IL-2 production decreased significantly on day 7 and 14 after cessation of benzene administration, but recovered with time (43, 71, and 79% of control on day 7, 14, and 21, respectively). Spleen cell number decreased significantly on the 7th day, but recovered on day 14 and 21. Female B6C3F$_1$ mice were exposed to 0, 12, 195, or 350 mg/kg/day benzene in drinking water for 30 days (Shell 1992). Decreased leukocytes were observed at 12 mg/kg/day, and decreased spleen cell number was observed at 195 mg/kg/day.

In the NTP-sponsored intermediate-duration oral study using Fischer 344 rats and B6C3F$_1$ mice, dose-related leukopenia and lymphopenia were observed for both male and female Fischer 344 rats at 200 and 600 mg/kg/day killed on day 60, and at all doses in female rats killed on day 120 (NTP 1986). Decreased

leukocytes were observed in male and female rats exposed for 60 days to 200 and 600 mg/kg/day benzene. Lymphoid depletion in the B-cells of the spleen was observed in animals exposed to 200 mg/kg/day (3 of 5 males, 4 of 5 females) and 600 mg/kg/day (5 of 5 males, 5 of 5 females) benzene for 60 days and in animals that received 600 mg/kg/day (10 of 10 males, 10 of 10 females) benzene for 120 days. At 600 mg/kg/day benzene exposure, increased extramedullary hematopoiesis was observed in the spleen of 4 of 5 male and 3 of 5 female rats. Dose-related leukopenia and lymphopenia were observed for both male and female mice exposed for 120 days, but not for 60 days. Leukocytes and lymphocytes were significantly decreased in male mice exposed for 120 days to 50, 100, 200, 400, and 600 mg/kg/day benzene. At 120 days of exposure, leukocytes were significantly decreased in female mice at 600 mg/kg/day and lymphocytes at 400 and 600 mg/kg/day. Histological examination revealed no adverse effects in mandibular lymph node or the thymus for either rats or mice (Huff et al. 1989; NTP 1986). Rats exposed to benzene at 50 and 100 mg/kg/day for 6 months had significant leukopenia (Wolf et al. 1956).

Leukopenia and lymphopenia were observed in mice at 31.5 mg/kg/day after 4 weeks of oral exposure (Hsieh et al. 1990). Reduction in thymus mass, suppression of B- and T-cell mitogenesis, and suppressed IL-2 release were also noted. Similar results were noted at 40 mg/kg/day (Hsieh et al. 1991). Oral administration of benzene to $B6C3F_1$ mice and Fischer 344 rats at doses of 50–200 mg/kg/day (male rats) or 25–100 mg/kg/day (female rats and male and female mice), 5 days/week for 103 weeks resulted in significant leukocytopenia and lymphocytopenia in both species (Huff et al. 1989; NTP 1986). In the thymus, lymphoid depletion was observed at 0, 10, 20, or 28% in male rats treated with 0, 50, 100, or 200 mg/kg/day, respectively. Increased incidences of lymphoid depletion of the spleen were observed in male rats treated with 0 (0%), 50 (40%), 100 (17%), and 200 (49%) mg/kg/day and in female rats treated with 0 (0%), 25 (22%), 50 (16%), and 100 (20%) mg/kg/day. In mice, an increased incidence of hematopoietic hyperplasia was observed in the bone marrow of dosed animals in both sexes. Splenic hematopoiesis was increased in dosed animals of both sexes of mice (Huff et al. 1989; NTP 1986). Maltoni et al. (1983, 1985) observed decreased leukocytes in Sprague-Dawley rats dosed with 500 mg/kg/day benzene for 84 weeks or more.

The highest NOAEL values and all reliable LOAEL values for immunologic effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.4 Neurological Effects

In humans, symptoms of central nervous system toxicity (including euphoria, vertigo, muscular incoordination, and unconsciousness) have been reported following one-time ingestion of benzene at 125 mg/kg (Thienes and Haley 1972).

Neurochemical profiles were conducted on rats after oral exposure to benzene (Kanada et al. 1994). Sprague-Dawley rats received a single dose of 950 mg/kg benzene by gavage and were sacrificed 2 hours after treatment. The control group received nothing. Brains were dissected into small-brain areas and stored until analysis. Acetylcholine, 3,4-dihydroxyphenylalanine (DOPA), dopamine, 3,4-dihydroxy-phenylacetic acid (DOPAC), homovanillic acid (HVA), norepinephrine, 3-methoxy-4-hydroxy-phenylglycol (MHPG), serotonin, and 5-hydroxyindoleacetic acid (5HIAA) contents in the small-brain regions were measured. Results showed that benzene decreased acetylcholine content of rat hippocampus. DOPA and norepinephrine content decreased in the rat midbrain. Dopamine, serotonin and 5HIAA content increased in the rat midbrain. Dopamine, DOPAC, norepinephrine, and 5HIAA content increased and serotonin content decreased in the rat hypothalmus after oral administration of benzene. Increased dopamine, HVA, MHPG, and serotonin content of rat medulla oblongata was observed. Decreased norepinephrine and 5HIAA content of rat medulla oblongata by benzene treatment was observed.

Oral exposure to benzene induced both synthesis and catabolism of monoamine neurotransmitters in CD-1 mice (Hsieh et al. 1988a). Mice given 8, 40, or 180 mg/kg/day of benzene for 4 weeks in drinking water exhibited changes in the levels of norepinephrine, dopamine, serotonin, and catecholamine metabolites in several brain regions but no treatment-related behavioral changes. Similar results were seen at 31.5–40 mg/kg/day (Hsieh et al. 1990, 1991). Because of the lack of association with behavioral changes, the effects on the neurotransmitters cannot be adequately assessed. Female $B6C3F_1$ mice were exposed to 0, 12, 195, or 350 mg/kg/day benzene in drinking water for 30 days (Shell 1992). Decreased brain weight was observed at 350 mg/kg/day. Sprague-Dawley rats given one oral dose of 88 mg/kg benzene exhibited slight central nervous system depression, whereas at 1,870 mg/kg/day, tremors were observed (Cornish and Ryan 1965). Histological examination of the brain revealed no treatment-related lesions after gavage treatment of male and female Fischer 344 rats and $B6C3F_1$ mice with doses up to 600 mg/kg/day for 120 days (NTP 1986). In the same experiment, $B6C3F_1$ mice exhibited tremors intermittently at doses of 400 mg/kg/day, which were more pronounced in males during the last 3 weeks of the study. No adverse effects based on histological examination of brain or spinal cord were observed

in male and female Fischer 344 rats and B6C3F$_1$ mice after chronic oral exposure to 50–200 mg/kg/day (male rats) or 25–100 mg/kg/day (female rats and male and female mice) (NTP 1986).

The highest NOAEL values and all reliable LOAEL values for neurological effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to benzene.

Female Sprague-Dawley rats were dosed by gavage with 0, 50, 250, 500, or 1,000 mg/kg benzene daily on Gd 6–15 and killed on Gd 20 (Exxon 1986). No adverse effects were noted on reproductive competency. No histological changes were reported in the prostate, testes, ovaries, mammary gland, or uterus of male and female Fischer 344 rats and B6C3F$_1$ mice dosed by gavage with up to 600 mg/kg/day benzene for 17 weeks (NTP 1986). In male and female Fischer 344 rats and B6C3F$_1$ mice after chronic oral exposure to 50–200 mg/kg/day (male rats) or 25–100 mg/kg/day (female rats and male and female mice), endometrial stromal polyps occurred with a significant positive trend in female rats (NTP 1986). The incidence in high dose group (14/50) was significantly greater than that in the control (7/50). In mice, analysis of preputial gland lesions in male mice dosed at 0, 25, 50, or 100 mg/kg showed increased incidences of focal, diffuse or epithelial hyperplasia (5, 65, 31, and 3%, respectively). The lower incidences of hyperplasia in the higher dose groups were probably due to the progression of the preputial gland lesions to neoplasias (see Section 3.2.2.7). Various non-neoplastic and neoplastic ovarian lesions were observed in dosed female mice, including epithelial hyperplasia and senile atrophy (NTP 1986).

The NOAEL and LOAEL values for reproductive effects in rats and mice are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans after oral exposure to benzene.

Benzene was embryotoxic as evidenced by reduced pup body weights when mice were administered 1,300 mg/kg/day of benzene by gavage on Gd 8–12 (Seidenberg et al. 1986). No maternal toxicity was observed. Female Sprague-Dawley rats were dosed by gavage with 0, 50, 250, 500, or 1,000 mg/kg

benzene daily on Gd 6–15 and killed on Gd 20 (Exxon 1986). No adverse effects were noted on morphological development.

The NOAEL value for rats and the LOAEL value for mice for developmental effects following acute oral exposure are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.7 Cancer

Essentially no information was located regarding the oral carcinogenicity of benzene in humans. Lymphatic and hematopoietic cancers were increased in vehicle maintenance workers who occasionally siphoned gasoline by mouth (Hunting et al. 1995), but the skin and lungs were the main routes of exposure (see Section 3.2.3.7).

Benzene has been shown to be a multiple site carcinogen by the oral route in animals (Huff et al. 1989; Maltoni et al. 1983, 1985, 1989; NTP 1986). In bioassays conducted by the NTP, benzene in corn oil was administered groups of 50 F344/N rats and 50 B6C3F$_1$ of each sex by gavage on 5 days/week for 103 weeks (Huff et al. 1989; NTP 1986). The male rats were exposed to dose levels of 0, 50, 100, or 200 mg/kg/day, and female rats and mice of both sexes were exposed to 0, 25, 50, or 100 mg/kg/day. In the rats, benzene caused significantly increased incidences of Zymbal gland carcinomas in males at ≥100 mg/kg/day and females at ≥25 mg/kg/day, oral cavity squamous cell papillomas and carcinomas in males at ≥50 mg/kg/day and females at ≥25 mg/kg/day, and skin squamous cell papillomas and carcinomas in males at 200 mg/kg/day. In the mice, benzene mainly caused significantly increased incidences of malignant lymphomas in both sexes at ≥25 mg/kg/day, Zymbal gland carcinomas in males at ≥50 mg/kg/day and females at 100 mg/kg/day, lung alveolar/bronchiolar adenomas and carcinomas in males at ≥100 mg/kg/day and females at ≥50 mg/kg/day, Harderian gland adenomas in males at ≥25 mg/kg/day, preputial gland squamous cell carcinomas in males at ≥50 mg/kg/day, and mammary gland carcinomas in females at ≥50 mg/kg/day. NTP (1986) concluded that there was clear evidence of carcinogenicity of benzene in male and female F344/N rats and B6C3F$_1$ mice under the conditions of these studies.

Maltoni et al. (1983, 1985, 1989) assessed carcinogenicity in groups of 30–50 rats and mice of each sex that were exposed to benzene in olive oil by gavage on 4–5 days/week for up to 104 weeks and observed for life. Sprague-Dawley rats were exposed to 0, 50, or 250 mg/kg/day for 52 weeks or 0 or 500 mg/kg/day for 104 weeks; effects included increased incidences of Zymbal gland carcinomas in

females at ≥50 mg/kg/day, oral cavity carcinomas in females at 250 mg/kg/day and both sexes at 500 mg/kg/day, forestomach carcinomas in females at 500 mg/kg/day, nasal cavity and skin carcinomas in males at 500 mg/kg/day, and liver angiosarcomas in both sexes at 500 mg/kg/day. Wistar rats were exposed to 0 or 500 mg/kg/day for 104 weeks; effects included increased incidences of Zymbal gland, nasal cavity, and oral cavity carcinomas. Swiss mice were exposed to 0 or 500 mg/kg/day for 78 weeks; effects included increased incidences Zymbal gland carcinomas in males, mammary carcinomas in females, and lung adenomas in both sexes. RF/J mice were exposed to 0 or 500 mg/kg/day for 52 weeks; effects included increased incidences of mammary carcinomas in females and lung adenomas in both sexes.

As discussed in Section 3.2.1.7, there is a consensus that benzene is a human carcinogen (IARC 1987, 2004, 2007; IRIS 2007; NTP 2005). This conclusion is based on sufficient inhalation data in humans supported by animal evidence, including the oral studies summarized above. The human cancer induced by inhalation exposure to benzene is predominantly acute nonlymphocytic leukemia, whereas benzene is a multiple site carcinogen in animals by both the inhalation and oral routes. Due to the lack of oral carcinogenicity data in humans, as well as the lack of a well-demonstrated and reproducible animal model for leukemia from benzene exposure, EPA extrapolated an oral slope factor from the inhalation unit risk range (IRIS 2007). The oral slope factor ranges from $1.5x10^{-2}$ to $5.5x10^{-2}$ $(mg/kg/day)^{-1}$, and for cancer risks of $1x10^{-4}$–$1x10^{-7}$, the corresponding dose levels range from $6.7x10^{-3}$–$1.8x10^{-3}$ to $6.7x10^{-6}$–$1.8x10^{-6}$ mg/kg/day, respectively. These risk levels are presented in Figure 3-1.

3.2.3 Dermal Exposure

3.2.3.1 Death

No studies were located regarding deaths in animals after dermal exposure to benzene.

A cohort of 338 men was investigated as to causes of death among employees of the fleet maintenance division of Washington DC's Department of Public Works (Hunting et al. 1995). This mortality study was undertaken because of three cases of leukemia among car and mobile equipments mechanics. Preliminary evaluation showed that the garage mechanics regularly used gasoline to clean parts and wash their hands; these workers also experienced dermal and inhalation exposure to gasoline during maintenance of vehicles. The men were employed for at least 1 year between January 1, 1977, and December 31, 1989. Cause-specific SMRs were calculated. Increased risk of death was found in some categories.

3.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, musculoskeletal, hepatic, renal, endocrine, or body weight effects in humans or animals after dermal exposure to benzene. Available data pertaining to hematological, dermal, and ocular effects are presented below. All reliable LOAEL values for systemic effects in humans and rabbits for acute- and chronic-duration dermal exposure are recorded in Table 3-3.

Hematological Effects. Tondel et al. (1995) reported a case of myelofibrosis that was diagnosed in a 46-year-old man who had worked from 1962 to 1979 as a gasoline station attendant. Although the exposure was primarily by inhalation, it is likely that dermal exposure also occurred.

Dermal Effects. In humans, benzene is a skin irritant. By defatting the keratin layer, it may cause erythema, vesiculation, and dry and scaly dermatitis (Sandmeyer 1981). Acute fatal exposure to benzene vapors caused second degree burns on the face, trunk, and limbs of the victims (Avis and Hutton 1993). Fifteen male workers were exposed to benzene vapors (>60 ppm) over several days during the removal of residual fuel from shipyard fuel tanks (Midzenski et al. 1992). Exposures to benzene range from 1 day to 3 weeks (mean of 5 days), 2.5–8 hours/day (mean of 5.5 hours). Workers with more than 2 days (16 hours) exposure reported mucous membrane irritation (80%), and skin irritation (13%) after exposure to the vapor.

Benzene was slightly irritating to the skin of rabbits (Wolf et al. 1956). The skin showed moderate erythema, edema, and moderate necrosis following application 1 time/day for 4 weeks.

Ocular Effects. Solvent workers who were exposed to 33 (men) or 59 (women) ppm benzene exhibited eye irritation while being exposed to the vapors (Yin et al. 1987b).

A transient increase in lacrimation was observed in male rats exposed to 10–300 ppm benzene for 6 hours/day, 5 days/week (Shell 1980). Moderate conjunctival irritation and transient corneal damage were observed in rabbits subsequent to placement of 2 drops of benzene onto the eyeball (Wolf et al. 1956).

Table 3-3 Levels of Significant Exposure to Benzene - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL Less Serious		LOAEL Serious	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Systemic								
Human	1-21 d 2.5-8 hr/d	Dermal		60 M ppm	(mucous membrane anc skin irritation)		Midzenski et al. 1992	
Rabbit (NS)	once	Ocular		2 Unknown	(moderate conjunctival irritations; light corneal injury)		Wolf et al 1956	
INTERMEDIATE EXPOSURE								
Systemic								
Rat (CD)	10 wk 5 d/wk 6 hr/d	Ocular	1 M ppm	10 M ppm	(lacrimation)		Shell 1980	
CHRONIC EXPOSURE								
Systemic								
Human	>1 yr (occup)	Ocular		33 M ppm	(eye irritation)		Yin et al. 1987b	
				59 F ppm	(eye irritation)			

d = day(s); F = female; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; NS = not specified; occup = occupational; yr = year

3.2.3.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological and lymphoreticular effects in humans or animals after dermal exposure to benzene.

3.2.3.4 Neurological Effects

Tondel et al. (1995) reported the case of a gasoline station attendant who had worked from 1962 to 1979 and who developed myelofibrosis. The patient described symptoms of fatigue for 3 weeks and night sweats, among other symptoms. Although the exposure was primarily by inhalation, it is probable that dermal exposure also occurred.

No studies were located regarding neurological effects in animals after dermal exposure to benzene.

3.2.3.5 Reproductive Effects

No studies were located regarding reproductive effects in humans or animals after dermal exposure to benzene.

3.2.3.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals after dermal exposure to benzene.

3.2.3.7 Cancer

A cohort mortality study was conducted to estimate the relative risk of hematological cancer among 335 male vehicle maintenance workers who were employed for at least 1 year between 1977 and 1989 and followed through 1991 (Hunting et al. 1995). The workers were car and mobile equipment mechanics who regularly used gasoline to clean parts and wash their hands. Exposure to gasoline also occurred via inhalation, and some of the workers occasionally siphoned gasoline by mouth. Since most of the cohort consisted of non-white (race data were missing for 9.5% of the cohort) vehicle maintenance workers who lived within the District of Columbia, death rates for age- and calendar-matched non-white males in the District of Columbia were used to calculate expected deaths for the cohort. Three deaths due to lymphatic and hematopoietic cancer were observed, yielding SMRs of 3.63 (95% CI 0.75–10.63) in the whole cohort and 4.22 (95% CI 0.87–12.34) in a subgroup of 297 workers with the highest potential for

exposure. Neither of these SMRs were significant, although analysis by cancer subtype showed a significantly elevated risk for leukemia and aleukemia in the high exposure subgroup (two cases, SMR=9.26, 95% CI 1.12–33.43). Aleukemia refers to leukemias in which the blood has normal or near-normal white blood cell counts, but few numbers of young leukocytes. Two additional cases of leukemia were identified among workers who were not included in the cohort (one died after the end of the follow-up period and another was still alive). Mortalities from causes other than lymphatic/hematopoietic cancer were not significantly increased.

Application of benzene to the skin of animals has not produced evidence of carcinogenicity, although most studies were inadequate for evaluation. As summarized by IARC (1982, 1987), many dermal carcinogenicity studies of chemicals other than benzene used benzene as a vehicle, and treated large numbers of control animals (mice) with benzene alone. None of these studies indicated that benzene induced skin tumors; however, all possible tumor sites usually were not examined.

3.3 GENOTOXICITY

The genotoxic effects of benzene have been studied extensively. The *in vivo* and *in vitro* data are summarized in Tables 3-4 and 3-5, respectively. In chronically-exposed humans, benzene and/or its metabolites primarily cause chromosomal aberrations (Andreoli et al. 1997; Bogadi-Šare et al. 1997; Ding et al. 1983; Forni and Moreo 1967, 1969; Forni et al. 1971a; Hartwich et al. 1969; Hedli et al. 1991; Karacic et al. 1995; Kašuba et al. 2000; Major et al. 1992, 1994; Picciano 1979; Popp et al. 1992; Rothman et al. 1995; Sardas et al. 1994; Sasiadek et al. 1989; Sellyei and Kelemen 1971; Smith et al. 1998; Sul et al. 2002; Tompa et al. 1994; Tough and Court Brown 1965; Tough et al. 1970; Türkel and Egeli 1994; Van den Berghe et al. 1979; Yardley-Jones et al. 1990; Zhang et al. 1998b, 1999). Chromosomal aberrations in humans are frequently demonstrated in peripheral blood lymphocytes and bone marrow. Although inhalation, oral, and dermal routes are all potential pathways of exposure relevant to humans, available *in vivo* human data are usually drawn from occupational settings in which inhalation and dermal exposure routes are most prevalent. In most of these studies, chromosome abnormalities were detected in workers exposed to high concentrations of benzene, sufficient to produce blood dyscrasias. However, Qu et al. (2003a, 2003b) noted a concentration-related increase in chromosomal aberrations across a wide range of exposure concentrations, including workers with relatively low-level benzene exposure. Limitations of many of the occupational studies include lack of accurate exposure data, possible coexposure to other chemicals, and lack of appropriate control groups.

Table 3-4. Genotoxicity of Benzene *In Vivo*

Species (test system)	End point	Result	Reference
Prokaryotic cells:			
Escherichia coli (host mediated DNA repair)	DNA synthesis	–	Hellmér and Bolcsfoldi 1992a
Invertebrate animal cells:			
Drosophila melanogaster	Sex-linked recessive lethal	–	Kale and Baum 1983
Spermatocytes	Recombination	–	
Spermatogonia	Recombination	+	
Spermatocytes	Heritable translocation	–	
Mammalian cells:			
Mouse (bone marrow)	Chromosomal aberrations	+	Giver et al. 2001; Shelby and Witt 1995; Siou et al. 1981
Mouse (bone marrow)	Chromosomal aberrations	+[a]	Meyne and Legator 1980
Mouse (bone marrow)	Chromosomal aberrations	(+)	Tice et al. 1980; 1982
Mouse (spleen lymphocytes)	Chromosomal aberrations	+	Au et al. 1991; Rithidech et al. 1987
Mouse (lymphoid cells, myeloid cells)	Chromosomal aberrations	+	Giver et al. 2001
Rat (bone marrow)	Chromosomal aberrations	+	Fujie et al. 1990; Hoechst 1977; Philip and Jensen 1970; Styles and Richardson 1984
Rat (bone marrow)	Chromosomal aberrations	+[b]	Anderson and Richardson 1981[b]
Rat (bone marrow)	Chromosomal aberrations	–	Hoechst 1977
Chinese hamster (bone marrow)	Chromosomal aberrations	+	Siou et al. 1981
Rabbit (bone marrow)	Chromosomal aberrations	+	Kissling and Speck 1972; 1973
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	+	Bogadi-Šare et al. 1997; Ding et al. 1983; Forni et al. 1971a; Kašuba et al. 2000; Picciano 1979; Sasiadek 1992; Sasiadek and Jagielski 1990; Sasiadek et al. 1989; Smith et al. 1998; Tompa et al. 1994; Tough and Court Brown 1965; Tough et al. 1970; Zhang et al. 1998a, 1999
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	(+)	Yardley-Jones et al. 1990
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	–	Bogadi-Šare et al. 1997; Jablonická et al. 1987
Mouse (bone marrow)	Micronuclei	+	Shelby and Witt 1995; Shelby et al. 1993
Mouse (bone marrow PCEs)	Micronuclei	+[c]	Ciranni et al. 1988
Mouse[d] (bone marrow PCEs)	Micronuclei	+[e]	Suzuki et al. 1989

Table 3-4. Genotoxicity of Benzene *In Vivo*

Species (test system)	End point	Result	Reference
Mouse (bone marrow PCEs)	Micronuclei	+	Au et al. 1990; Barale et al. 1985; Chen et al. 1994; Diaz et al. 1980; Erexson et al. 1986; Farris et al. 1996; Harper et al. 1984; Hite et al. 1980; Siou et al. 1981; Toft et al. 1982
Mouse (bone marrow PCEs)	Micronuclei	+[a]	Meyne and Legator 1980
Mouse (bone marrow PCEs)	Micronuclei	+[f]	Eastmond et al. 2001
Mouse (bone marrow NCEs)	Micronuclei	+	Farris et al. 1996
Mouse (bone marrow NCEs)	Micronuclei	+[f]	Eastmond et al. 2001
Mouse (pregnant/bone marrow PCEs)	Micronuclei	(+)	Ciranni et al. 1988
Mouse (peripheral blood)	Micronuclei	+	Hayashi et al. 1992; Healy et al. 2001
Mouse (peripheral blood PCEs)	Micronuclei	+	Farris et al. 1996
Mouse (peripheral blood PCEs)	Micronuclei	+[c,g]	Luke et al. 1988a
Mouse (peripheral blood NCEs)	Micronuclei	+	Barale et al. 1985; Choy et al. 1985; Farris et al. 1996; Rithidech et al. 1988
Mouse (peripheral blood NCEs)	Micronuclei	+[c,f]	Luke et al. 1988a
Mouse (lung fibroblasts)	Micronuclei	+	Ranaldi et al. 1998
Mouse (fetus/liver cells)	Micronuclei	+	Ciranni et al. 1988
Rat (lymphocytes)	Micronuclei	+	Erexson et al. 1986
Chinese hamster (bone marrow)	Micronuclei	+	Siou et al. 1981
Human (lymphocytes)	Micronuclei	+	Robertson et al. 1991
Human (occupational exposure/lymphocytes)	Micronuclei	+	Liu et al. 1996
Human (occupational exposure/lymphocytes)	Micronuclei	–	Pitarque et al. 1996; Surrallés et al. 1997
Mouse (bone marrow)	Sister chromatid exchange	+	Tice et al. 1980; 1982
Mouse (pregnant/bone marrow)	Sister chromatid exchange	+	Sharma et al. 1985
Mouse (lymphocytes)	Sister chromatid exchange	+	Erexson et al. 1986
Mouse (fetus/liver cells)	Sister chromatid exchange	+	Sharma et al. 1985
Rat (lymphocytes)	Sister chromatid exchange	+	Erexson et al. 1986
Human (occupational exposure/lymphocytes)	Sister chromatid exchange	+	Popp et al. 1992
Human (occupational exposure/lymphocytes)	Sister chromatid exchange	–	Kašuba et al. 2000; Pitarque et al. 1997; Seiji et al. 1990; Yardley-Jones et al. 1988
Mouse (spleen lymphocytes)	Mutations	+	Ward et al. 1992
Mouse (lung tissue)	Mutations	+	Mullin et al. 1998
Mouse embryo (premelanocytes)	Mutations (deletions)	+	Schiestl et al. 1997
Human (bone marrow)	Mutations (gene-duplicating)	+	Rothman et al. 1995

Table 3-4. Genotoxicity of Benzene *In Vivo*

Species (test system)	End point	Result	Reference
Human (bone marrow)	Mutations (gene-inactivating)	–	Rothman et al. 1995
Mouse (bone marrow)	DNA adducts	+	Arfellini et al. 1985; Creek et al. 1997; Lévay et al. 1996; Pathak et al. 1995; Turteltaub and Mani 2003
Mouse (white blood cells)	DNA adducts	+	Lévay et al. 1996
Mouse (liver)	DNA adducts	+	Arfellini et al. 1985; Creek et al. 1997; Mani et al. 1999; Turteltaub and Mani 2003
Rat (bone marrow)	DNA adducts	+	Arfellini et al. 1985; Creek et al. 1997; Lévay et al. 1996; Pathak et al. 1995; Turteltaub and Mani 2003
Rat (liver)	DNA adducts	+	Arfellini et al. 1985; Creek et al. 1997; Lutz and Schlatter 1977; Mani et al. 1999; Mazzullo et al. 1989; Turteltaub and Mani 2003
Mouse (peripheral blood lymphocytes)	DNA strand breaks	+	Tuo et al. 1996
Rat (lymphocytes, bone marrow, spleen, liver)	DNA strand breaks	+	Lee et al. 2005
Human (occupational exposure/lymphocytes)	DNA strand breaks	+	Andreoli et al. 1997; Nilsson et al. 1996; Sul et al. 2002
Mouse (peripheral blood lymphocytes, bone marrow)	DNA damage	+	Chang et al. 2005
Human (occupational exposure/lymphocytes)	DNA repair efficiency	_	Hallberg et al. 1996
Rat (bone marrow)	DNA oxidative damage	+	Kolachana et al. 1993
Human (occupational exposure/lymphocytes)	DNA oxidative damage	+	Liu et al. 1996
Mouse (bone marrow)	DNA synthesis inhibition	+	Lee et al. 1988
Rabbit (bone marrow)	DNA synthesis inhibition	+	Kissling and Speck 1972
Mouse (bone marrow)	RNA synthesis inhibition	+	Kissling and Speck 1972
Rat (liver mitochondria)	RNA synthesis inhibition	+	Kalf et al. 1982

Table 3-4. Genotoxicity of Benzene *In Vivo*

Species (test system)	End point	Result	Reference
Mouse (spermatogonia)	Sperm head abnormality	+	Topham 1980

[a]This result was observed following both oral and intraperitoneal exposure.
[b]This result was observed following both inhalation and intraperitoneal exposure.
[c]Males affected to a significantly greater degree than females.
[d]Two strains of mouse were tested; Ms/Ae and CD-1. The result applies to both strains.
[e]This result was observed following both oral and intraperitoneal exposure; however, oral exposure produced the greater effect.
[f]Increase in micronuclei was exposure duration-dependent.
[g]Increase in micronuclei was exposure duration-independent.

+ = Positive result; – = negative result; (+) = weakly positive result; DNA = deoxyribonucleic acid; NCEs = normochromatic erythrocytes; PCEs = polychromatic erythrocytes; RNA = ribonucleic acid

Table 3-5. Genotoxicity of Benzene *In Vitro*

Species (test system)	End point	Result: With activation	Result: Without activation	Reference
Prokaryotic organisms:				
Salmonella typhimurium (Ames test)	Gene mutation	–	–	De Flora et al. 1984
S. typhimurium (histidine reversion)	Gene mutation	+	–	Glatt et al. 1989
S. typhimurium (azaquanine reversion)	Gene mutation	+	No data	Kaden et al. 1979; Seixas et al. 1982
Bacillus subtilis (histidine reversion)	Gene mutation	–	–	Tannoka 1977
Escherichia coli (DNA polymerase 1/cell-free DNA synthetic system)	DNA synthesis inhibition	No data	–	Lee et al. 1988
E. coli (host meditated DNA repair)	DNA synthesis	No data	No data	Hellmér and Bolcsfoldi 1992b
Plasmid DNA ΦX-174 RF I	DNA degradation	No data	+	Li et al. 1995
Eukaryotic organisms:				
Fungi:				
Aspergillus nidulans (methionine supressors)	Gene mutation	No data	–	Crebelli et al. 1986
Mammalian cells:				
Mouse (L5178Y cells/TK test)	Gene mutation	–	–	Oberly et al. 1984
Chinese hamster (ovary cell culture)	Chromosomal aberrations	–	–	Gulati et al. 1989
Human (lymphocyte cell culture)	Chromosomal aberrations	No data	+	Eastmond et al. 1994; Morimoto 1976
Human (lymphocyte cell culture)	Chromosomal aberrations	No data	–	Gerner-Smidt and Friedrich 1978
Human (lymphoblastoid culture)	Intrachromosomal recombination	No data	+	Aubrecht et al. 1995
Chinese hamster (ovary cell culture)	Micronuclei	–	–	Douglas et al. 1985
Human (whole blood cells)	Micronuclei	–	–	Zarani et al. 1999
Chinese hamster (ovary cell culture)	Sister chromatid exchange	–	–	Douglas et al. 1985; Gulati et al. 1989
Human (lymphocyte cell culture)	Sister chromatid exchange	+	No data	Morimoto 1983
Human (lymphocyte cell culture)	Sister chromatid exchange	No data	–	Gerner-Smidt and Friedrich 1978
Rabbit (bone marrow mitoplasts)	DNA adducts	No data	+	Rushmore et al. 1984

Table 3-5. Genotoxicity of Benzene *In Vitro*

Species (test system)	End point	Result: With activation	Result: Without activation	Reference
Rat (liver mitoplasts)	DNA adducts	No data	+	Rushmore et al. 1984
Calf thymus DNA	DNA adducts	No data	+	Chenna et al. 1995
Human (bone marrow)	DNA adducts	No data	+	Bodell et al. 1993; Lévay and Bodell 1992
Human (leukemia cells)	DNA adducts	No data	+	Bodell et al. 1993; Lévay and Bodell 1992
Rat (hepatocytes)	DNA breaks	No data	–	Bradley 1985
Chinese hamster (ovary cell culture)	DNA breaks	+	+	Douglas et al. 1985
Chinese hamster (ovary cell culture)	DNA breaks	+	+[a]	Lakhanisky and Hendricks 1985
Chinese hamster (V79 cell culture)	DNA breaks	–	–	Swenberg et al. 1976
Mouse (L5178Y cell culture)	DNA breaks	No data	–	Pellack-Walker and Blumer 1986
Human (leukemia cells)	DNA oxidative damage	No data	+	Kolachana et al. 1993
Human (lymphocyte cell culture)	DNA repair	No data	_	Hallberg et al. 1996
Rat liver epithelial cells	DNA hyperphos-phorylatioin	No data	+	Dees and Travis 1994
Rat (hepatocyte culture)	Unscheduled DNA synthesis	No data	(+)	Glauert et al. 1985
Rat (hepatocyte culture)	Unscheduled DNA synthesis	No data	–	Probst and Hill 1985; Williams et al. 1985
Human (HeLa S3 cells)	Unscheduled DNA synthesis	–	–	Barrett 1985
Mouse (bone marrow cell culture)	DNA synthesis inhibition	No data	+	Lee et al. 1988
Mouse (bone marrow cell culture)	DNA synthesis inhibition	+	(+)	Lee et al. 1989
Calf (thymus DNA polymerase α/cell-free DNA synthetic system)	DNA synthesis inhibition	No data	+	Lee et al. 1988
Human (HeLa cells)	DNA synthesis inhibition	–	–	Painter and Howard 1982
Mouse (spleen lymphocytes)	RNA synthesis inhibition	No data	+	Post et al. 1985

Table 3-5. Genotoxicity of Benzene *In Vitro*

Species (test system)	End point	Result		Reference
		With activation	Without activation	
Rat (liver mitoplasts)	RNA synthesis inhibition	No data	+	Kalf et al. 1982
Cat, rabbit (bone marrow mitoplasts)	RNA synthesis inhibition	No data	+	Kalf et al. 1982

[a]Benzene's effect on DNA breaks was reduced when metabolic activators were used.

– = negative results; + = positive results; (+) = weakly positive result; DNA = deoxyribonucleic acid; RNA = ribonucleic acid

Chromosomal aberrations observed in workers chronically exposed to benzene include hypo- and hyperdiploidy, deletions, breaks, and gaps. For example, analysis of peripheral lymphocytes of workers exposed to benzene vapors at a mean concentration of 30 ppm revealed significant increases in monosomy of chromosomes 5, 7, and 8 (but not 1), and tri- and/or tetrasomy of chromosomes 1, 5, 7, and 8 (Zhang et al. 1998b, 1999). In another series of epidemiological studies in workers chronically exposed to benzene, nonrandom effects were apparent in chromosomes 1, 2, 4, and 9; nonrandom breaks in chromosomes 2, 4, and 9 were twice as prevalent in benzene-exposed workers versus controls; and chromosomes 1 and 2 were nearly twice as prone to gaps (Sasiadek and Jagielski 1990; Sasiadek et al. 1989). Twenty-one people with hematological signs of chronic benzene poisoning exhibited significantly more chromosomal abnormalities than controls (Ding et al. 1983). A significant increase in dicentric chromosomes and unstable aberrations was noted in 36 female workers exposed to benzene in a shoe factory for up to 32 years (Kašuba et al. 2000). Significant increases in hyperploidy of chromosomes 8 and 21 and translocations between chromosomes 8 and 21 were observed in workers exposed to benzene vapors at a mean TWA of 31 ppm (Smith et al. 1998).

DNA repair efficiency was evaluated in blood lymphocytes collected from exposed or unexposed workers in a petrochemical plant (Hallberg et al. 1996). Plasmids ($pCMV_{CAT}$) were irradiated with UV light (254 nm) to induce thymidine dimers and were then transfected into blood lymphocytes from workers. Transfected plasmids that were repaired in the lymphocytes would express the chloramphenicol actetyltransferase reporter gene (CAT) product whereas unrepaired plasmids would not. Lymphocytes from exposed or unexposed workers did not show significant differences in their ability to repair light-damaged DNA; however, the authors suggest that the sample population was too small to detect any differences given the large individual variations in repair capacity (Hallberg et al. 1996).

Oxidative DNA damage was assessed in workers (n=87) exposed to benzene by conducting measurements of 8-hydroxy-2-deoxyguanosine (8-OHdG) in peripheral blood lymphocytes (Liu et al. 1996). 8-OHdG is formed by hydroxy radical (OH·) addition at the C-8 position of deoxyguanosine (Kasai and Nishimura 1986) and appears to be a biomarker of oxidative DNA damage (Liu et al. 1996). The exposure to benzene was classified as low, medium, or high (mean benzene levels of 2.46, 103, or 424 mg/m^3, respectively [corresponding to 0.78, 32.2, or 133 ppm]). Levels of 8-OHdG in exposed workers rose in a concentration-related manner although the increases were significant only in the medium and high exposure groups. Toluene, also detected in the workplace air, did not alter levels of 8-OHdG. Formation of micronuclei, a measure of DNA damage, increased in a concentration-related manner to levels that were significantly higher than those in controls. The levels of urinary *trans,trans*-

muconic acid, a biomarker of benzene exposure, were well correlated with levels of 8-OHdG which, in turn, were well correlated with levels of lymphocyte micronuclei. The findings of Liu et al. (1996) are supported by the results of a study by Nilsson et al. (1996) in which concentration-related increased urinary levels of 8-OHdG were reported in male workers (n=30) at a gasoline station. Breathing zone benzene concentrations ranged from 0.003 to 0.6 ppm (mean 0.13 ppm). Significant concentration-related increases in DNA single-strand breaks were also noted in these workers. Collectively, the results of Liu et al. (1996) and Nilsson et al. (1996) provide suggestive evidence that benzene metabolites may induce reactive oxygen species, which could result in oxidative DNA damage and formation of hydroxylated bases such as 8-OHdG (Liu et al. 1996).

Rothman et al. (1995) used the glycophorin A (GPA) gene loss mutation assay to assess the nature of DNA damage in workers heavily exposed to benzene. The GPA assay measures the frequency of variant cells that have lost the expression of the M form of the GPA gene in the peripheral blood of heterozygous (MN) subjects. The variant cells possess the NN phenotype (double-copy expression of the N allele and no expression of M) or the NØ phenotype (single-copy expression of the N allele and no expression of M). The NN variants are thought to arise from mitotic recombination, chromosome loss, and reduplication, or gene conversion. The NØ variants appear to be associated with point mutations, deletions, or gene inactivation. Rothman et al. (1995) demonstrated a significant increase in the frequency of NN cells in benzene-exposed workers (compared with unexposed control subjects) in the absence of a significant effect on the frequency of NØ cells. These results suggest that benzene induces gene-duplicating, but not gene-inactivating, mutations at the GPA locus in benzene-exposed humans.

Sister chromatid exchange was not found to be a significant effect of benzene exposure in humans (Kašuba et al. 2000; Seiji et al. 1990; Yardley-Jones et al. 1988); however, the selection of control subjects in the studies by Seiji et al. (1990) and Yardley-Jones et al. (1988) was poor. Refer to Table 3-4 for a further summary of these results.

In vivo animal studies provide convincing evidence of benzene's genotoxicity (Table 3-4). Furthermore, the finding that male mice are more sensitive than females to benzene-induced chromosomal damage is consistent among reports (Armstrong and Galloway 1993; Barale et al. 1985; Choy et al. 1985; Ciranni et al. 1988; Hatakeyama et al. 1992; Meyne and Legator 1980; Siou et al. 1981). Consistently positive findings for chromosomal aberrations in bone marrow and lymphocytes in animals support the human case reports and epidemiological studies in which chromosomal damage was linked to benzene exposure. Micronucleus assays are popular methods for crudely analyzing DNA damage in animals. Positive results

were observed in all studies testing for increased micronuclei frequencies. One of these micronucleus assays (Luke et al. 1988a) investigated the effects of different inhalation exposure durations on polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) in peripheral blood. PCEs are newly formed erythrocytes that contain mRNA and as a result, exhibit staining when RNA staining reagents are used. NCEs are mature erythrocytes that lack mRNA and are not stained under the same conditions. The researchers found that PCEs are good indicators of recent and acute exposure, while NCEs are good indicators of accumulated long-term exposure (Luke et al. 1988a). Although no human studies were located that reported increased sister chromatid exchange in exposed individuals, increases in sister chromatid exchange were reported in mice and rats (Erexson et al. 1986; Sharma et al. 1985; Tice et al. 1980, 1982). *In vivo*, *trans*,*trans*-muconaldehyde (a metabolite of benzene) has also been shown to induce highly significant increases in sister chromatid exchanges in mice (Witz et al. 1990a). In addition to oral and inhalation routes, many researchers tested subcutaneous and intraperitoneal routes as well; the results for these alternate routes of exposure were largely positive for chromosomal aberrations in bone marrow (Anderson and Richardson 1981; Kissling and Speck 1972, 1973; Kolachana et al. 1993; Meyne and Legator 1980; Philip and Jensen 1970), micronuclei in bone marrow (Diaz et al. 1980), and sister chromatid exchange in mouse fetus liver cells (Sharma et al. 1985). Binding of benzene and/or its metabolites to DNA, RNA, and proteins has been consistently observed in rats and mice (Arfellini et al. 1985; Creek et al. 1997; Lévay et al. 1996; Mani et al. 1999; Mazullo et al. 1989; Turteltaub and Mani 2003). Arfellini et al. (1985) noted that binding to RNA and proteins was more prevalent than binding to DNA. Lévay et al. (1996) observed dose- and time-dependent formation of two DNA adducts in white blood cells and bone marrow cells of mice administered benzene for 7 days via intraperitoneal injection. Turteltaub and Mani (2003) consistently observed DNA and protein binding in mice at intraperitoneal doses as low as 5 μg/kg body weight. One study using intraperitoneal injections reported a dose-dependent increase in sperm head abnormalities in mice exposed to 0.5 or 0.6 mL benzene/kg/day (Topham 1980). The author views sperm head abnormality as a possible indication of heritable mutations. However, from this study alone, it cannot be determined if benzene causes such transmissible genetic mutations. For these and other results from animal *in vivo* studies, see Table 3-4.

Molecular mechanisms of benzene-induced genotoxicity have been studied to some extent in laboratory animals. Schiestl et al. (1997) noted benzene-induced reversion of the mouse pink-eye unstable mutation in premelanocytes of mouse embryos following intraperitoneal injection of the chemical into pregnant dams; the reversions resulted from genomic DNA deletions. Results of Chen et al. (1994) and Eastmond et al. (2001) indicate that benzene-induced micronuclei in mouse bone marrow erythrocytes are formed predominantly from chromosome breakage, but also from aneuploidy.

In vitro studies strongly imply that benzene's genotoxicity is derived primarily from its metabolites. Positive results were obtained for gene mutation in *Salmonella typhimurium* (Glatt et al. 1989; Kaden et al. 1979; Seixas et al. 1982) and sister chromatid exchange in human lymphocyte cell culture (Morimoto 1983) only when exogenous metabolic activators of benzene were used. Similarly, endogenous metabolic activation was required for effects to be seen on DNA synthesis in rat hepatocyte culture (Glauert et al. 1985), DNA adduct formation in rat liver mitoplasts (Rushmore et al. 1984), and RNA synthesis in rat liver mitoplasts and in rabbit and cat bone marrow mitoplasts (Kalf et al. 1982). Endogenous activation occurs naturally by enzymes already within the cells. Exogenous activation requires the addition of enzymes to cellular preparations. In one study using benzene and 13 possible metabolites, Glatt et al. (1989) found that *trans*-1,2-dihydrodiol (with metabolic activators) and the diol epoxides (with or without metabolic activators) produced histidine reversion in *S. typhimurium*. The same researchers also investigated the genotoxicity of these 13 proposed metabolites in V79 Chinese hamster cells; *anti*-diol epoxide, *syn*-diol epoxide, 1,2,3-trihydroxybenzene, 1,2,4-trihydroxybenzene, quinone, hydroquinone, catechol, phenol, and 1,2-dihydrodiol were found to produce genotoxic effects ranging from sister chromatid exchange and micronuclei increases to gene mutations (Glatt et al. 1989). Similar studies with *trans,trans*-muconaldehyde showed that this metabolite is strongly mutagenic in V79 cells and weakly mutagenic in bacteria (Glatt and Witz 1990). Chang et al. (1994) showed that muconaldehyde and its aldehyde metabolites 6-hydroxy-*trans,trans*-2,4-hexadienal and 6-oxo-*trans,trans*-hexadienoic acid were mutagenic in V79 cells.

Several studies revealed a possible connection between certain benzene metabolites and DNA damage via the formation of oxygen radicals. Lewis et al. (1988) found that both 1,2,4-benzenetriol and hydroquinone produce DNA strand breaks, 1,2,4-benzenetriol to a greater degree than hydroquinone. Consistent with these findings was the observation that 1,2,4-benzenetriol generates a greater concentration of oxygen radicals than hydroquinone. The study concluded that 1,2,4-benzenetriol damages DNA by producing oxygen radicals, while hydroquinone probably exerts its genotoxic effects by some other mechanism (Lewis et al. 1988). However, results of Li et al. (1995b) indicate that much of the benzene-mediated DNA damage may result from the generation of reactive oxygen species via a copper-redox cycling mechanism involved in the oxidation of the benzene metabolite, hydroquinone. Increased recombination, which can lead to adverse genetic changes, was observed in Chinese hamster ovary cells exposed to phenol, catechol, or benzoquinone (Winn 2003). Benzene had no effect on recombination. The observed increases in recombination were abolished by the addition of catalase to the cells, suggesting that the effect of the benzene metabolites was elicited by oxidative stress (Winn 2003).

Oxidative damage (manifested as DNA strand breaks) was observed in HL60 cells treated with 1,4-benzoquinone or 1,4-hydroquinone (Hiraku and Kawanishi 1996). 1,4-Benzoquinone and hydroquinone induced DNA strand breaks in Chinese hamster ovary cells while phenol, catechol, 1,2,4-benzenetriol, *trans,trans*-muconic acid, and *S*-phenyl mercapturic acid did not (Sze et al. 1996). No synergism between hydroquinone and the other metabolites was observed.

Phenol, catechol, hydroquinone, and benzene induced morphological transformation and gene mutations in Syrian hamster embryo cells (Tsutsui et al. 1997). Chromosomal aberrations, sister chromatid exchange, and unscheduled DNA synthesis were increased by the benzene metabolites while aneuploidy was observed in cells treated with benzene or catechol (Tsutsui et al. 1997). There was no significant increase ($p>0.005$) in DNA fragmentation in human respiratory epithelial cells exposed to an atmosphere of 5 mg benzene/m^3 for 8 hours, although there was evidence of an inflammatory response (Gosepath et al. 2003). Chromosomal breaks and hyperdiploidy were observed in human lymphocytes after exposure to hydroquinone *in vitro* (Eastmond et al. 1994). Aneusomy of chromosomes 7 and 8 were observed in human umbilical cord blood cells treated with hydroquinone (2, 10, or 50 μM) for 72 hours (Smith et al. 2000). Monosomy 7 and trisomy 8 are two common clonal aberrations observed in myeloid leukemias (Smith et al. 2000). Hydroquinone (26–49 μM) also induced monosomy of chromosomes 5, 7, and 8 in a human lymphoblast cell line (Stillman et al. 1997). Hydroquinone (10–100 μM) and 1,2,4-benzenetriol (10–50 μM) significantly increased monosomy 5 and 7 in human lymphocytes and long-arm deletions in chromosomes 5 and 7 (Zhang et al. 1998b). Benzene metabolites have been shown to form DNA adducts in human bone marrow and HL-60 cells (Bodell et al. 1993; Lévay and Bodell 1992). Zhang et al. (1993) showed that 1,2,4-benzenetriol increased the frequency of micronuclei formation in human lymphocytes in culture, and in HL60 cells in a dose-related manner. An increase in the level of oxidative damage to DNA was also noted in HL60 cells in culture. Extracts from human cells have been shown to have repair activity toward benzoquinone-DNA adducts *in vitro* (Chenna et al. 1995). Benzene (1–5 mM) did not elicit micronuclei formation in whole blood cells treated for 48 hours with or without metabolic activation with 10% S9 rat liver fraction for 2 hours (Zarani et al. 1999). The assay was conducted with blood collected from four subjects. Chen and Eastmond (1995) showed that benzene metabolites can adversely affect human topoisomerases, enzymes involved in DNA replication and repair. No effect of any metabolite was seen on human topoisomerase I or for topoisomerase II for hydroquinone, phenol, 2,2'-biphenol, 4,4'-biphenol, and catechol at concentrations as high as 500 μM. 1,4-Benzoquinone and 1,2,4-benzenetriol inhibited human topoisomerase II *in vitro*, at 500 and 250 μM without bioactivation. However, following bioactivation, phenol and 2,2'-biphenol showed inhibitory effects at doses as low as 50 μM, whereas 4,4'-biphenol inhibited topoisomerase II at concentrations of 10 μM. More recently,

Eastmond et al. (2001) demonstrated decreased activity of topoisomerase II activity in nucleated bone marrow cells of mice administered benzene by oral gavage for subchronic durations.

Available *in vitro* data suggest that benzene itself is genotoxic. Two studies reported that benzene produced DNA breaks in Chinese hamster ovary cells independent of metabolic activators (Douglas et al. 1985; Lakhanisky and Hendrickx 1985). In a study by Aubrecht et al. (1995), benzene was shown to induce intrachromosomal recombination in human lymphoblastoid cell culture. Therefore, benzene appears to have some genotoxic capabilities of its own, but its metabolites seem to be the primary genotoxins in systems in which normal metabolism is occurring. Refer to Table 3-5 for the results of these and other *in vitro* studies.

In summary, chromosome aberrations have been found consistently in bone marrow cells of persons occupationally exposed to benzene. The conclusion, based on human epidemiological studies, that benzene is a human clastogen is well supported by *in vivo* animal studies and *in vitro* cell cultures and subcellular studies. Virtually all studies that looked for effects at the chromosomal level were positive when the ability to metabolize benzene was present. These experimental results are consistent with the chromosomal damage seen in exposed humans. The leukemia observed in some benzene-exposed persons may result from the appearance of a clone of chromosomally abnormal cells in the bone marrow. With respect to genetic effects, no safe human exposure level can be determined from available epidemiological data. Significant increases in sister chromatid exchanges were produced in bone marrow cells and lymphocytes of animals. The significance of sister chromatid exchanges is unknown, but their production by a chemical is generally considered to indicate a genotoxic potential. Exposures generally occur via inhalation, and based on animal studies, effects following oral exposure may be greater than effects following inhalation exposure to comparable levels of benzene. Data presented in this section and elsewhere in this profile (Section 3.4) show that benzene metabolites are the genotoxic entities. It is possible that each metabolite causes a different genotoxic effect. Differences in metabolic capability are probably responsible for some of the variations in response to benzene seen in different test systems.

3.4 TOXICOKINETICS

The toxicokinetics of benzene has been extensively studied. Inhalation exposure is probably the major route of human exposure to benzene, although oral and dermal exposure are also important. Benzene is readily absorbed following inhalation or oral exposure. Although benzene is also readily absorbed from the skin, a significant amount of a dermal application evaporates from the skin surface. Absorbed

benzene is rapidly distributed throughout the body and tends to accumulate in fatty tissues. The liver serves an important function in benzene metabolism, which results in the production of several reactive metabolites. Although it is widely accepted that benzene toxicity is dependent upon metabolism, no single benzene metabolite has been found to be the major source of benzene hematopoietic and leukemogenic effects. At low exposure levels, benzene is rapidly metabolized and excreted predominantly as conjugated urinary metabolites. At higher exposure levels, metabolic pathways appear to become saturated and a large portion of an absorbed dose of benzene is excreted as parent compound in exhaled air. Benzene metabolism appears to be qualitatively similar among humans and various laboratory animal species. However, there are quantitative differences in the relative amounts of benzene metabolites.

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

Inhalation exposure is probably the major route of human exposure to benzene, and numerous studies of absorption of benzene after inhalation exposure in different settings have been conducted (Ashley et al. 1994; Avis and Hutton 1993; Boogaard and van Sittert 1995; Brunnemann et al. 1989; Byrd et al. 1990; Etzel and Ashley 1994; Fustinoni et al. 1995; Ghittori et al. 1995; Gordian and Guay 1995; Hajimiragha et al. 1989; Hanzlick 1995; Karacic et al. 1995; Kok and Ong 1994; Lagorio et al. 1994a; Laitinen et al. 1994; Lauwerys et al. 1994; Lindstrom et al. 1994; Mannino et al. 1995; Nomiyama and Nomiyama 1974a; Ong et al. 1994, 1995; Pekari et al. 1992; Popp et al. 1994; Rauscher et al. 1994; Rothman et al. 1995; Ruppert et al. 1995; Scherer et al. 1995; Shamy et al. 1994; Srbova et al. 1950; Yu and Weisel 1996). Existing evidence indicates that benzene is rapidly absorbed by humans following inhalation exposure. Results from a study of 23 subjects who inhaled 47–110 ppm benzene for 2–3 hours showed that absorption was highest in the first few minutes of exposure, but decreased rapidly thereafter (Srbova et al. 1950). In the first 5 minutes of exposure, absorption was 70–80%, but by 1 hour, it was reduced to approximately 50% (range, 20–60%). Respiratory uptake (the amount of benzene absorbed from the lungs following inhalation of the vapors) in six volunteers including males and females exposed to 52–62 ppm benzene for 4 hours was determined to be approximately 47%(Nomiyama and Nomiyama 1974a). In a similar study, three healthy nonsmoking volunteers were exposed to benzene at levels of 1.6 or 9.4 ppm for 4 hours (Pekari et al. 1992). The amount of benzene absorbed was estimated from the difference between the concentration inhaled and the concentration exhaled. Estimates were 48% for the high dose and 52% for the low dose, supporting the evidence of Nomiyama and Nomiyama (1974a). Yu and Weisel (1996) measured the uptake of benzene by three female subjects exposed to benzene in smoke

generated by burning cigarettes, which resulted in airborne benzene concentrations in the range of 32–69 ppm. Average absorption for exposure periods of 30 or 120 minutes was 64% and did not appear to be influenced by exposure duration.

Studies of occupational exposure to benzene suggest that absorption occurs both by inhalation and dermally in many workplace settings. In a study conducted in 1992 in Finland, car mechanics' exposure to benzene was evaluated (Laitinen et al. 1994). Different work phases were measured at five Finnish garages. Blood samples from car mechanics (eight nonsmokers) were taken 3–9 hours after exposure to benzene. The results were approximated to the time point of 16 hours after exposure. Fourteen air samples were taken from the breathing zone and five stationary samples were collected from the middle of the garage for background concentration levels. The average background concentration (stationary samples) of gasoline vapors was 6±7 cm^3/m^3 (2±2 ppm) and the concentration of benzene was under the detection limit of 0.2 cm^3/m^3 (0.1 ppm). The concentrations of benzene in the breathing zone varied from the detection limit of 0.2 cm^3/m^3 to 1.3 cm^3/m^3 (0.1–0.4 ppm) for unleaded gasoline and from the detection limit to 3.7 cm^3/m^3 (1.2 ppm) for leaded gasoline. The highest benzene exposure level (2.4–3.7 cm^3/m^3 or 0.8–1.2 ppm) was measured when changing the filter to the fuel pump. The mechanics worked without protective gloves, and the risk of contamination and penetration through the skin was significant. During carburetor renewal and gathering, benzene concentrations were 0.5–1.1 cm^3/m^3 (0.2–0.3 ppm). During changing of the fuel filter to electronic fuel-injection system, benzene concentration ranged from 0.9 to 3.4 cm^3/m^3 (0.3–1.1 ppm). The approximated benzene concentrations in blood corresponding to the time point of 16 hours after the exposure showed much higher levels of exposure than could be expected according to the corresponding air measurements (8-hour TWA). The comparison of expected benzene concentrations in blood, if no dermal exposure were present, to the levels at the time point of 16 hours after the exposure showed that the dermal route must be the source of about 68% of exposure (range 1.1–88.2%). Two of eight workers had minimal exposure through the skin (0–1.1%). The other six workers showed high dermal exposure (79.4%).

Exposure to benzene-contaminated water can also provide an opportunity for both inhalation and dermal absorption. In a series of experiments conducted in a single-family residence from June 11 to 13, 1991, exposure to benzene through contaminated residential water was monitored (Lindstrom et al. 1994). The residential water was contaminated with benzene and other hydrocarbons in 1986. Periodic testing conducted from 1986 to 1991 showed benzene concentrations ranging from 33 to 673 μg/L (ppb). The experiment involved an individual taking a 20-minute shower with the bathroom door closed, followed by 5 minutes for drying and dressing; then the bathroom door was opened and this individual was allowed to

leave the house. Integrated 60- and 240-minute whole-air samples were collected from the bathroom, an adjacent bedroom, living room, and in ambient air. Glass, gas-tight syringe grab samples were simultaneously collected from the shower, bathroom, bedroom, and living room at 0, 10, 18, 20, 25, 25.5, and 30 minutes. Two members of the monitoring team were measured for 6 hours using personal Tenax gas GC monitors. For the first 30 minutes of each experiment, one member was based in the bathroom and the other in the living room. Benzene concentrations in the shower head ranged from 185 to 367 μg/L (ppb), while drain level samples ranged from below the detectable limit (0.6 μg/L or ppb) to 198 μg/L (ppb). Analysis of the syringe samples suggested a pulse of benzene moving from the shower stall to the rest of the house over approximately 60 minutes. Peak levels of benzene measured 758–1,670 $\mu g/m^3$ (235–518 ppb) in the shower stall at 18–20 minutes, 366–498 $\mu g/m^3$ (113–154 ppb) in the bathroom at 10–25 minutes, 81–146 $\mu g/m^3$ (25–45 ppb) in the bedroom at 25.5–30 minutes, and 40–62 $\mu g/m^3$ (12–19 ppb) in the living room at 36–70 minutes. The individual who took the 20-minute shower had estimated inhalation doses of 79.6, 105, and 103 μg (mean=95.9 μg) for the 3 consecutive sampling days. These doses were estimated by taking the products of the concentration of benzene in water, the minute ventilation rate, the duration of exposure, and a 70% benzene absorption factor. This was 2.1–4.9 times higher than corresponding 20-minute bathroom exposures. Adding the average dose absorbed in the bathroom during the 5.5 minutes following the shower (using the overall 20–25 minutes mean syringe level of 318 $\mu g/m^3$ [99 ppb]) gave a total average shower-related inhalation dose of 113 μg. An average dermal dose of 168 μg was estimated for the 20-minute shower by multiplying the average concentration of benzene in water by the surface area of the male volunteer, an exposure factor of 75% body surface area exposed, a dermal permeability constant for benzene of 0.11 cm/hour, an exposure duration of 0.33 hours, and a unit conversion factor of 11/1,000 cm^2. The total benzene dose resulting from the shower was estimated to be approximately 281 μg (40% via inhalation and 60% via dermal), suggesting a higher potential exposure to benzene via dermal contact from the water than through vaporization and inhalation. This exposure was 2–3.5 times higher than the mean 6-hour inhalation dose received by the sampling members. The estimated inhalation and dermal doses reported by Lindstrom et al. (1994) have not been validated by others and are therefore of questionable value for quantitative analysis.

Additional evidence of benzene absorption following inhalation exposure comes from data on cigarette smokers. Benzene levels were significantly higher in the venous blood of 14 smokers (median level of 493 ng/L) than in a control group of 13 nonsmokers (median level of 190 ng/L) (Hajimiragha et al. 1989). Cigarette smoke is known to contain benzene (Brunnemann et al. 1989; Byrd et al. 1990), and the subjects had no known exposure to other sources of benzene (Hajimiragha et al. 1989). Kok and Ong (1994)

report blood and urine levels of benzene as 110.9 and 116.4 ng/L, respectively for nonsmokers, and 328.8 and 405.4 ng/L, respectively for smokers. The National Association of Medical Examiners Pediatric Toxicology (PedTox) Registry reported blood benzene concentrations ranging from 0.2 to 4.9 mg/L in eight children who died in fires and were dead at the scene, indicating absorption of benzene from burning materials (Hanzlick 1995). Blood benzene levels taken from U.S. engineers (Group I) and firefighters (Group II) working at burning oil wells in Kuwait were compared to blood benzene levels from non-exposed U.S. citizens (Etzel and Ashley 1994). The median concentrations of benzene in whole blood from Groups I, II, and U.S. reference group were 0.035 μg/L (range ND–0.055 μg/L), 0.18 μg/L (range 0.063–1.1 μg/L), and 0.066 μg/L (range ND–0.54 μg/L), respectively. The median concentration in group II was generally higher than the median concentrations in group I or the reference group. Statistically significant higher concentrations of benzene ($p<0.0001$) were found in group II smokers than in Group II nonsmokers.

Animal data confirm that benzene is rapidly absorbed through the lungs. Inhalation studies with laboratory dogs indicate that distribution of benzene throughout the animal's body is rapid, with tissue values dependent on blood supply. A linear relationship existed between the concentration of benzene in air (200–1,300 ppm) and the equilibrium concentration in blood (Schrenk et al. 1941). At these exposures, the concentrations of benzene in the blood of dogs exposed to benzene reached a steady state within 30 minutes.

In rodents, the extent of uptake increased linearly with concentration for exposures up to 200 ppm. At concentrations of >200 ppm, zero-order kinetics were observed (i.e., uptake became nonlinear, indicating saturation of the metabolic capacity). The percentage of inhaled benzene that was absorbed and retained during a 6-hour exposure period decreased from 33 to 15% in rats and from 50 to 10% in mice as the exposure concentration was increased from about 10 to 1,000 ppm (Sabourin et al. 1987). When rats and mice were exposed to approximately 300 ppm, mice had greater uptake than rats. Mice and rats had different absorption characteristics; the cumulative inhaled dose in mice was greater than that in rats (Eutermoser et al. 1986; Sabourin et al. 1987). Purebred Duroc-Jersey pigs were exposed to 0, 20, 100, and 500 ppm benzene vapors 6 hours/day, 5 days/week for 3 weeks (Dow 1992). The average concentration of phenol in the urine increased linearly with dose.

3.4.1.2 Oral Exposure

Although definitive scientific data are not available on oral absorption of benzene in humans, case studies of accidental or intentional poisoning indicate that benzene is absorbed by the oral route (Thienes and Haley 1972).

Benzene appears to be efficiently absorbed following oral dosing in animals. Oral absorption of benzene was first demonstrated by Parke and Williams (1953a). After radiolabeled (^{14}C) benzene was administered orally to rabbits (340–500 mg/kg), the total radioactivity eliminated in exhaled air and urine accounted for approximately 90% of the administered dose, indicating that at least this much of the administered dose was absorbed. Studies in rats and mice showed that gastrointestinal absorption was greater than 97% in both species when the animals were administered benzene by gavage (in corn oil) at doses of 0.5–150 mg/kg/day (Sabourin et al. 1987). In many animal studies, benzene is administered orally in oil to insure predictable solubility and dose concentration control. This is unlike the predicted human oral exposure, which is likely to be in drinking water. There are a number of studies in which benzene has been administered to animals in the drinking water, which more closely resembles predicted human oral exposure (Lindstrom et al. 1994). Although no information was located regarding the extent of oral absorption of benzene in aqueous solutions, it is reasonable to assume that oral absorption from water solutions would be nearly 100%.

The bioavailability of pure as opposed to soil-adsorbed benzene was conducted in adult male rats (Turkall et al. 1988). Animals were gavaged with an aqueous suspension of benzene alone, or adsorbed to clay or sandy soil. Plasma concentration, half-life, tissue distribution, respiratory excretion, and urinary excretion were monitored. Peak plasma concentration of radioactivity was increased in the presence of either soil as opposed to benzene alone, while sandy soil also decreased the time to peak plasma concentration as opposed to benzene alone. Soil increased the area under the plasma radioactivity-time curve as opposed to benzene alone, a difference that was significant with clay soil. The half-life in plasma was not affected by soil.

3.4.1.3 Dermal Exposure

Studies conducted *in vivo* in humans and *in vitro* using human skin indicates that benzene can be absorbed dermally. The movement of a substance through the skin to the blood occurs by passive diffusion and has been described mathematically by Fick's law. However, this is an oversimplification of

the process of skin absorption; various factors (e.g., interaction of benzene with molecules within the skin) affect the transport of the solvent through the skin (Lodén 1986).

In vivo experiments on four volunteers, to whom 0.0026 mg/cm^2 of ^{14}C-benzene was applied to forearm skin, indicated that approximately 0.05% of the applied dose was absorbed (Franz 1984). Absorption was rapid, with more than 80% of the total excretion of the absorbed dose occurring in the first 8 hours after application. Calculations were based on urinary excretion data and no correction was made for the amount of benzene that evaporated from the applied site before absorption occurred. In addition, the percentage of absorbed dose excreted in urine that was used in the calculation was based only on data from rhesus monkeys and may not be accurate for humans. In another study, 35–43 cm^2 of the forearm was exposed to approximately 0.06 g/cm^2 of liquid benzene for 1.25–2 hours (Hanke et al. 1961). The absorption was estimated from the amount of phenol eliminated in the urine. The absorption rate of liquid benzene by the skin (under the conditions of complete saturation) was calculated to be low, approximately 0.4 mg/cm^2/hour. The absorption due to vapors in the same experiment was negligible. The results indicate that dermal absorption of liquid benzene is of concern, while dermal absorption from vapor exposure may not be of concern because of the low concentration of benzene in vapor form at the point of contact with the skin. No signs of acute intoxication due to liquid benzene dermally absorbed were noted. These results confirm that benzene can be absorbed through skin. However, non-benzene-derived phenol in the urine was not accounted for.

Studies of occupational exposure to benzene suggest that absorption occurs both by inhalation and dermally in many workplace settings. In a study conducted in 1992 in Finland, car mechanics' exposure to benzene was evaluated (Laitinen et al. 1994). Different work phases were measured at five Finnish garages. Blood samples from car mechanics (eight nonsmokers) were taken 3–9 hours after exposure to benzene. The results were approximated to the time point of 16 hours after exposure. Fourteen air samples were taken from the breathing zone and five stationary samples were collected from the middle of the garage for background concentration levels. The mechanics worked without protective gloves, and the risk of contamination and penetration through the skin was significant. The approximated benzene concentrations in blood corresponding to the time point of 16 hours after the exposure showed much higher levels of exposure than could be expected according to the corresponding air measurements (8 hour TWA). The comparison of expected benzene concentrations in blood, if no dermal exposure was present, to the levels at the time point of 16 hours after the exposure showed that the dermal route must be the source of about 68% of exposure (range 1.1–88.2%). Two of eight workers had minimal exposure through the skin (0–1.1%). The other six workers showed high dermal exposure (79.4%).

Exposure to benzene-contaminated water can also provide an opportunity for both inhalation and dermal absorption. In a series of experiments conducted in a single-family residence from June 11 to 13, 1991, exposure to benzene through contaminated residential water was monitored (Lindstrom et al. 1994). The residential water was contaminated with benzene and other hydrocarbons in 1986. Exposure was monitored for a person taking a 20-minute shower and for people in other parts of the house during and after the shower. An average dermal dose of 168 μg was estimated for a 20-minute shower using this water. The total benzene dose resulting from the shower was estimated to be approximately 281 μg (40% via inhalation and 60% via dermal), suggesting a higher potential exposure to benzene via dermal contact from the water than through vaporization and inhalation (see Section 3.4.1.1 for a more detailed discussion). This exposure was 2–3.5 times higher than the mean 6-hour inhalation dose received by the sampling team members in other parts of the house. The estimated inhalation and dermal doses reported by Lindstrom et al. (1994) have not been validated by others and are therefore of questionable value for quantitative analysis.

In vitro experiments using human skin support the fact that benzene can be absorbed dermally. An experiment on the permeability of excised human skin with regard to benzene (specific activity 99.8 mCi/mmol; total volume of applied benzene not reported) resulted in the absorption of 0.17 mg/cm^2 after 0.5 hours and 1.92 mg/cm^2 after 13.5 hours (Lodén 1986). Following application of 5, 120, 270, and 520 μL/cm^2 of benzene to human skin, total absorption was found to be 0.01, 0.24, 0.56, and 0.9 μL/cm^2, respectively. Thus, the total amount absorbed appears to increase linearly with dose. The study author indicated that evaporation of benzene did not exceed 5%. When exposure time (i.e., the time to complete evaporation) at each dose was measured and plotted as the ordinate of absorption, total absorption was found to increase linearly with exposure time. The percentage of the applied dose absorbed at each concentration was constant at about 0.2% (Franz 1984).

Using results from an *in vitro* study, it was estimated that an adult working in ambient air containing 10 ppm benzene would absorb 7.5 μL/hour from inhalation and 1.5 μL/hour from whole-body (2 m^2) dermal exposure (Blank and McAuliffe 1985). It was also estimated that 100 cm^2 of smooth and bare skin in contact with gasoline containing 5% benzene would absorb 7.0 μL/hour. Diffusion through the stratum corneum was considered the most likely rate-limiting step for dermal absorption because of benzene's low water solubility (Blank and McAuliffe 1985).

Based on an observational study of workers in a tire factory, it was estimated that a worker exposed to benzene as a result of direct skin contact with petroleum naphtha containing 0.5% benzene could absorb 4–8 mg of benzene per day through intact skin (Susten et al. 1985). This amount absorbed was compared with an estimated 14 mg of benzene absorbed as a result of inhalation of 1 ppm for an 8-hour day. The estimate for dermal absorption is theoretical since in many facilities the concentration of benzene in rubber solvents such as petroleum naphtha is less than 0.5% and may be as low as 0.09%.

Benzene is also absorbed dermally by animals. In Rhesus monkeys, minipigs, and hairless mice, dermal absorption was <1% following a single direct (unoccluded) application of liquid benzene (Franz 1984; Maibach and Anjo 1981; Susten et al. 1985). As with humans, absorption appeared to be rapid, with the highest urinary excretion of the absorbed dose observed in the first 8 hours following exposure (Franz 1984). Multiple applications, as well as application to stripped skin, resulted in greater skin penetration (Maibach and Anjo 1981). The percentage of absorption of the applied dose of benzene in each of these animals was approximately 2–3-fold higher than that of humans.

Data indicate that soil adsorption decreases the dermal bioavailability of benzene. A study in which male rats were treated dermally with 0.004 mg/cm^2 ^{14}C-benzene, with or without 1 g of clay or sandy soil, reported benzene absorption half-lives of 3.1, 3.6, and 4.4 hours for pure benzene, sandy soil, and clay soil, respectively (Skowronski et al. 1988).

Benzene in air was rapidly absorbed through the skin of hairless mice that were attached to respirators to avoid pulmonary uptake of the benzene vapors (Tsuruta 1989). The rate of absorption of benzene through the skin increased linearly with dose. The skin absorption rate for 200 ppm was 4.11 nmol/cm^2/hour (0.31 μg/cm^2/hour); at 1,000 ppm, the rate was 24.2 nmol/cm^2/hour (1.89 μg/cm^2/hour), and at 3,000 ppm, the rate was 75.5 nmol/cm^2/hour (5.90 μg/cm^2/hour). The skin absorption coefficient was 0.619 cm/hour.

McDougal et al. (1990) estimated permeability constants of 0.15 and 0.08 cm/hour for rat and human skin, respectively, based on the appearance of benzene in the blood of rats dermally exposed to benzene vapors at a concentration of 40,000 ppm for 4 hours. A physiologically based pharmacodynamic (PBPK) model was used to estimate the permeability of the vapor in rat and human skin. These results indicate that dermal absorption of benzene may be greater in rats than humans. Therefore, results in rats may provide a conservative estimate of dermal absorption of benzene in humans.

In an *in vitro* experiment using Fischer 344 rat skin, the partition coefficient for skin:air was determined for benzene at 203 ppm (Mattie et al. 1994). The partition coefficient of a chemical in skin is an indicator of the capacity of the skin to retain the chemical, and may reflect the rate at which a chemical is absorbed through the skin and enters the circulation. Results indicated a partition coefficient of 35, with an equilibration time of 4 hours. The skin:air partition coefficient is necessary for developing the dermal compartment of a PBPK model.

Based on data for skin absorption of benzene vapors in mice and occupational exposure data, Tsuruta (1989) estimated the ratio of skin absorption rate to pulmonary uptake for humans exposed to benzene to be 0.037. Dermal absorption could account for a relatively higher percentage of total benzene uptake in occupational settings where personnel, using respirators but not protective clothing, are exposed to high concentrations of benzene vapor.

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

Information on the distribution of benzene in humans comes primarily from case studies. The data suggest that benzene is distributed throughout the body following absorption into blood. Since benzene is lipophilic, a high distribution to fatty tissue might be expected. Following inhalation exposure to benzene, the chemical has been detected in the biological fluids and tissues of the subjects (Pekari et al. 1992; Tauber 1970; Winek and Collom 1971; Winek et al. 1967). Fluid and tissue levels of benzene have been reported in cases of both accidental and intentional lethal exposures. Levels of 0.38 mg% in blood (mg% = mg per 100 mL of blood or mg per 100 g of tissue), 1.38 mg% in the brain, and 0.26 mg% in the liver were reported in a worker who died from exposure to very high air concentrations of the chemical (Tauber 1970). An autopsy (time after death not indicated) performed on a youth who died while sniffing reagent-grade benzene revealed benzene concentrations of 2.0 mg% in blood, 3.9 mg% in brain, 1.6 mg% in liver, 1.9 mg% in kidney, 1 mg% in stomach, 1.1 mg% in bile, 2.23 mg% in abdominal fat, and 0.06 mg% in urine (Winek and Collom 1971). Benzene crosses the human placenta and is present in the cord blood in amounts equal to or greater than those in maternal blood (Dowty et al. 1976). Benzene is expected to readily bind to plasma proteins (Travis and Bowers 1989). Furthermore, benzene metabolites have been found to form covalent adducts with proteins from blood in humans (Bechtold et al. 1992b; Rappaport et al. 2002a, 2002b; Yeowell-O'Connell et al. 1998) and mice (McDonald et al. 1994). The relatively widespread distribution of benzene and its metabolites to other tissues and organs indicates that

protein adduct formation in the blood does not adversely affect distribution, although no confirming studies were located.

Results from animal studies indicate that absorbed benzene is distributed among several compartments. The parent compound is preferentially stored in the fat, although the relative uptake in tissues also appears to be dependent on the perfusion rate of tissues by blood.

Following a 10-minute inhalation exposure of pregnant mice to 2,000 ppm benzene, parent compound and its metabolites were found to be present in lipid-rich tissues, such as brain and fat, and in well-perfused tissues, such as liver and kidney. Benzene was also found in the placenta and fetuses immediately following inhalation of benzene (Ghantous and Danielsson 1986). During inhalation exposure of rats to 500 ppm, benzene levels reached a steady-state concentration within 4 hours in blood (11.5 μg/mL), 6 hours in fat (164.4 μg/g), and less than 2 hours in bone marrow (37.0 μg/g) (Rickert et al. 1979). Benzene was also distributed to the kidney, lung, liver, brain, and spleen. The benzene metabolites phenol, catechol, and hydroquinone were detected in blood and bone marrow following 6 hours of exposure to benzene, with levels in bone marrow exceeding the respective levels in blood. The levels of phenol in blood and bone marrow decreased much more rapidly after exposure ceased than did those of catechol or hydroquinone, suggesting the possibility of accumulation of the latter two compounds.

Benzene was rapidly distributed throughout the bodies of dogs exposed via inhalation to concentrations of 800 ppm for up to 8 hours/day for 8–22 days (Schrenk et al. 1941). Fat, bone marrow, and urine contained about 20 times the concentration of benzene in blood; benzene levels in muscles and organs were 1–3 times that in blood; and erythrocytes contained about twice the amount of benzene found in plasma. During inhalation exposure of rats to 1,000 ppm (2 hours/day, for 12 weeks), benzene was stored longer (and eliminated more slowly) in female and male rats with higher body fat content than in leaner animals (Sato et al. 1975).

Benzene was detected in the liver, lung, and blood of rats and mice examined immediately following a 6-hour exposure to benzene vapors at a concentration of 50 ppm (Sabourin et al. 1988). Sabourin and coworkers (Sabourin et al. 1987, 1988) also examined effects exposure concentration, exposure rate, and route of administration on the comparative metabolism of benzene in rats and mice. Results of these studies are summarized in Section 3.4.3 (Metabolism).

3.4.2.2 Oral Exposure

No studies were located regarding distribution in humans after oral exposure to benzene.

In Sprague-Dawley rats administered a single dose of 0.15, 1.5, 15, 150, or 500 mg/kg of ^{14}C-benzene by gavage, benzene was rapidly absorbed and distributed to various organs and tissues within 1 hour of administration (Low et al. 1989). One hour after rats were dosed with 0.15 or 1.5 mg/kg of benzene, tissue distribution of benzene was highest in liver and kidney, intermediate in blood, and lowest in the Zymbal gland, nasal cavity tissue, and mammary gland. At higher doses, beginning with 15 mg/kg, benzene disproportionately increased in the mammary glands and bone marrow. Bone marrow and adipose tissue proved to be depots of benzene at the higher dose levels. The highest tissue concentrations of benzene's metabolite hydroquinone 1 hour after administration of 15 mg/kg of benzene were in the liver, kidney, and blood, while the highest concentrations of the metabolite phenol were in the oral cavity, nasal cavity, and kidney. The major tissue sites of benzene's conjugated metabolites were blood, bone marrow, oral cavity, kidney, and liver for phenyl sulfate and hydroquinone glucuronide; muconic acid was also found in these sites. Additionally, the Zymbal gland and nasal cavity were depots for phenyl glucuronide, another conjugated metabolite of benzene. The Zymbal gland is a specialized sebaceous gland and a site for benzene-induced tumors. Therefore, it is reasonable to expect that lipophilic chemicals like benzene would partition readily into this gland. However, benzene did not accumulate in the Zymbal gland; within 24 hours after administration, radiolabel derived from ^{14}C-benzene in the Zymbal gland constituted less than 0.0001% of the administered dose.

The bioavailability of pure as opposed to soil-adsorbed benzene was conducted in adult male rats (Turkall et al. 1988). Animals were gavaged with an aqueous suspension of ^{14}C-benzene alone, or adsorbed to clay or sandy soil. Two hours after exposure, stomach tissue contained the highest amount of radioactivity, followed by fat in all treatment groups. No differences in tissue distribution patterns were detected for the three treatments.

3.4.2.3 Dermal Exposure

No studies were located regarding distribution in humans after dermal exposure to benzene.

A study of male rats treated dermally with 0.004 mg/cm^2 of ^{14}C-benzene, with and without 1 g of clay or sandy soil, revealed soil-related differences in tissue distribution following treatment. The ^{14}C activity (expressed as a percentage of initial dose per g of tissue) 48 hours after treatment with soil-adsorbed

benzene was greatest in the treated skin (0.059–0.119%), followed by the kidney (0.024%) and liver (0.013–0.015%), in both soil groups. In the pure benzene group, the kidney contained the largest amount of radioactivity (0.026%), followed by the liver (0.013%) and treated skin (0.11%) (Skowronski et al. 1988). In all three groups, <0.01% of the radioactivity was found in the following tissues: duodenum, fat, bone marrow, esophagus, pancreas, lung, heart, spleen, blood, brain, thymus, thyroid, adrenal, testes, untreated skin, and remaining carcass.

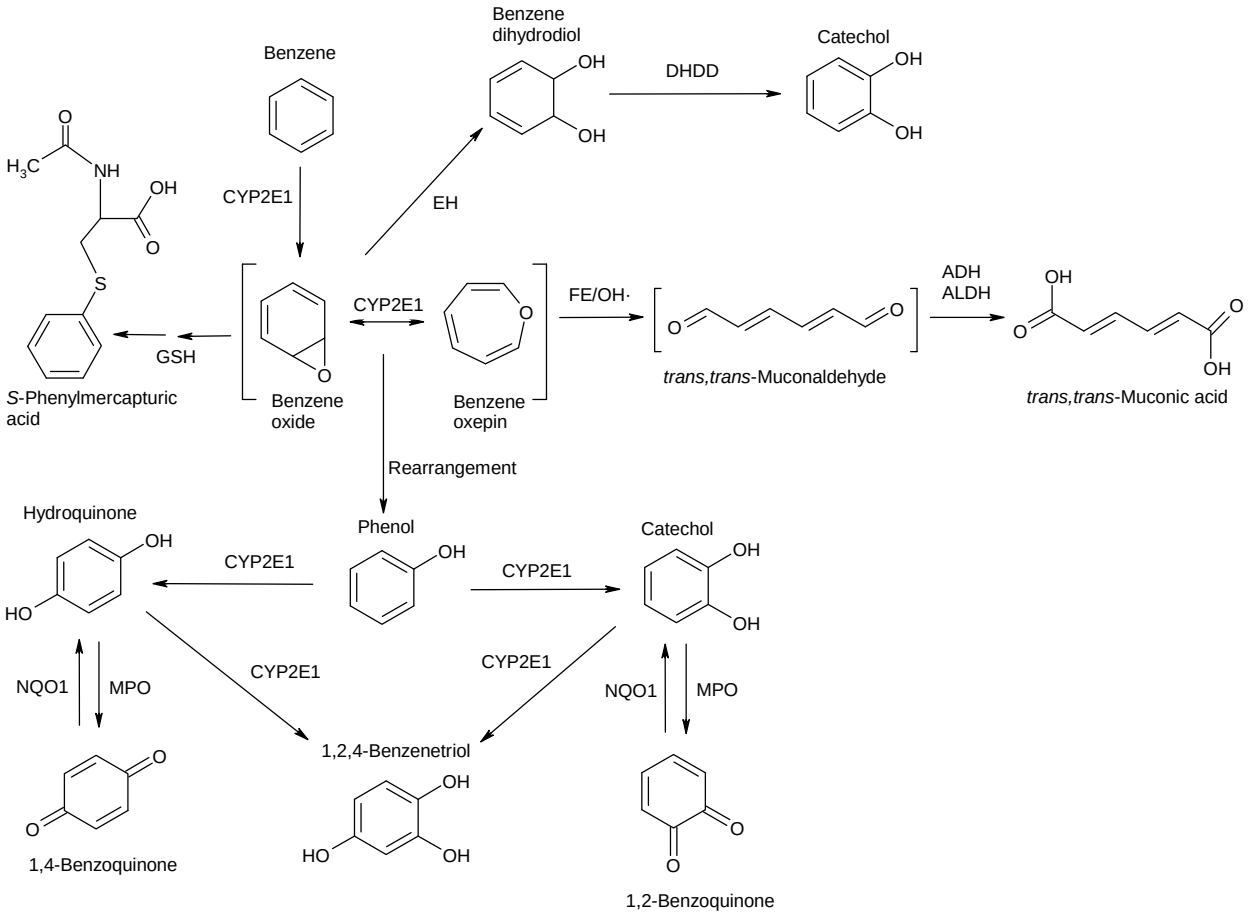
3.4.3 Metabolism

Although the metabolism of benzene has been studied extensively, the steps leading to benzene toxicity are not yet fully understood. It is generally understood that both cancer and noncancer effects are caused by one or more reactive metabolites of benzene. Available data indicate that metabolites produced in the liver are carried to the bone marrow where benzene toxicity is expressed. Benzene metabolism may occur, at least in part, in the bone marrow. Benzene metabolism has been demonstrated in isolated perfused rabbit lung preparations (Powley and Carlson 2002). As discussed in detail in Section 3.5, available evidence suggests that multiple benzene metabolites may collectively be responsible for the expression of benzene toxicity.

Data regarding metabolism of benzene in humans are derived primarily from studies using inhalation exposures. Benzene is excreted both unchanged via the lungs and as metabolites (but also as parent compound in small amounts) in the urine. The rate and percentage of excretion via the lungs are dependent on exposure dose and route. Qualitatively, the metabolism and elimination of benzene appear to be similar in humans and laboratory animals, but no directly comparable studies are available (Henderson et al. 1989; Sabourin et al. 1988).

The metabolic scheme shown in Figure 3-3 is based on results of numerous mechanistic studies of benzene metabolism (see Henderson et al. 1989; Huff et al. 1989; and Ross 1996, 2000 for comprehensive reviews of benzene metabolism). The first step is the cytochrome P-450 2E1 (CYP2E1) catalyzed oxidation of benzene to form benzene oxide (Lindstrom et al. 1997), which is in equilibrium with its oxepin (Vogel and Günther 1967). Several pathways are involved in the metabolism of benzene oxide. The predominant pathway involves nonenzymatic rearrangement to form phenol (Jerina et al. 1968), the major initial product of benzene metabolism (Parke and Williams 1953a). Phenol is oxidized in the presence of CYP2E1 to catechol or hydroquinone, which are oxidized via myeloperoxidase (MPO) to the reactive metabolites 1,2- and 1,4-benzoquinone, respectively (Nebert et al. 2002). The reverse

Figure 3-3. Metabolic Pathways for Benzene



ADH = alcohol dehydrogenase; ALDH = aldehyde dehydrogenase; CYP2E1 = cytochrome P-450 2E1; DHDD = dihydrodiol dehydrogenase; EH = epoxide hydrolase; GSH = glutathione; MPO = myeloperoxidase; NQ01 = NAD(P)H:quinone oxidoreductase

Source: adapted from Nebert et al. 2002; Ross 2000

reaction (reduction of 1,2- and 1,4-benzoquinone to catechol and hydroquinone, respectively) is catalyzed by NAD(P)H:quinone oxidoreductase (NQ01) (Nebert et al. 2002). Both catechol and hydroquinone may be converted to the reactive metabolite 1,2,4-benzenetriol via CYP2E1 catalysis. Alternatively, benzene oxide may undergo epoxide hydrolase-catalyzed conversion to benzene dihydrodiol and subsequent dihydrodiol dehydrogenase-catalyzed conversion to catechol (Nebert et al. 2002; Snyder et al. 1993a, 1993b). Each of the phenolic metabolites of benzene (phenol, catechol, hydroquinone, and 1,2,4-benzenetriol) can undergo sulfonic or glucuronic conjugation (Nebert et al. 2002; Schrenk and Bock 1990); the conjugates of phenol and hydroquinone are major urinary metabolites of benzene (Sabourin et al. 1989a; Wells and Nerland 1991). Other pathways of benzene oxide metabolism include: (1) reaction with glutathione (GSH) to form S-phenylmercapturic acid (Nebert et al. 2002; Sabourin et al. 1988; Schafer et al. 1993; Schlosser et al. 1993; Schrenk et al. 1992; van Sittert et al. 1993), and (2) iron-catalyzed ring-opening conversion to *trans*,*trans*-muconic acid, presumably via the reactive *trans*,*trans*-muconaldehyde intermediate (Bleasdale et al. 1996; Nebert et al. 2002; Ross 2000; Witz et al. 1990b, 1990c, 1996).

Results of several studies provide strong evidence for the involvement of CYP2E1 in the oxidation of benzene. For example, no signs of benzene-induced toxicity were observed in transgenic CYP2E1 knockout mice (that do not express hepatic CYP2E1 activity) following exposure to benzene vapors (200 ppm, 6 hours/day for 5 days) that caused severe genotoxicity and cytotoxicity in wild-type mice (Valentine et al. 1996a, 1996b). Pretreatment of mice with CYP inhibitors (toluene, propylene glycol, β-diethyl amino ethyl diphenyl propyl acetate hydrogen chloride [SKF-525A]) has been demonstrated to reduce both benzene metabolite formation (Andrews et al. 1977; Gill et al. 1979; Ikeda et al. 1972; Tuo et al. 1996) and resulting genotoxicity (DNA damage as assessed by the alkaline comet assay) in mice (Tuo et al. 1996). Pretreatment with CYP inducers (3-methylcholanthrene and β-naphthoflavone) increased both benzene metabolism and benzene clastogenicity (Gad-El-Karim et al. 1986). Immunoinhibition studies in rat and rabbit hepatic microsomes provide additional support to the major role of CYP2E in benzene metabolism (Johansson and Ingelman-Sundberg 1988; Koop and Laethem 1992). Occupationally exposed workers with a phenotype corresponding to rapid CYP2E1 metabolism were more susceptible to benzene hematotoxicity than workers not expressing this phenotype (Rothman et al. 1997). *In vitro* studies using human liver microsomes demonstrate a positive correlation between benzene metabolism and CYP2E1 activity (Nedelcheva et al. 1999; Seaton et al. 1994). Although CYP2E1 appears to be the major catalyzing agent in initial benzene metabolism, other CYPs, such as CYP2B1 and CYP2F2, may also be involved (Gut et al. 1996a, 1996b; Powley and Carlson 2000, 2001; Sheets and Carlson 2004; Sheets et al. 2004; Snyder et al. 1993a, 1993b).

CYPs involved in benzene metabolism are found in all tissues. However, the predominant repository is the liver, which is considered to be the primary site of benzene metabolism. By demonstrating that partial hepatectomy diminished both the rate of metabolism of benzene and its toxicity in rats exposed to benzene via subcutaneous injection, Sammett et al. (1979) provided suggestive evidence that one or more benzene metabolites formed in the liver are necessary for toxicity. *In vitro* studies have demonstrated that pulmonary microsomes of humans and laboratory animals are capable of metabolizing benzene, which appears to be catalyzed by both CYP2E1 and CYP2F2 (Powley and Carlson 1999, 2000; Sheets et al. 2004). There is some indication that CYP2E1-catalyzed benzene metabolism may also occur in bone marrow, a major target tissue of benzene toxicity. Andrews et al. (1979) demonstrated that rabbit bone marrow is capable of metabolizing benzene. Schnier et al. (1989) subsequently found that rabbit bone marrow contains CYP2E1. Irons et al. (1980) demonstrated that benzene metabolism by rat bone marrow (*in situ*) was complete and independent of metabolism by the liver, with concentrations of phenol greater than catechol and hydroquinone. Although the total metabolism by bone marrow was limited (total metabolites present were 25% of those in blood), the concentration of metabolites in the bone marrow exceeded that in the blood. Similar studies have been conducted in mice (Ganousis et al. 1992). Fibroblasts had elevated levels of glutathione-S-transferase activity relative to macrophages, whereas macrophages had higher levels of UDP-glucuronyltransferase and peroxidase activity. These data suggest that cell-specific metabolism of benzene in the marrow may contribute to the toxicity of benzene in this tissue compartment. In addition, comparison of the detoxifying activities of rat and mouse bone marrow stromal cells indicates that rats have higher levels of glutathione and quinone reductase, which are known to play critical roles in modulating hydroquinone-induced toxicity; this suggests a metabolic basis for the observed increased susceptibility of mice to benzene-induced hematotoxicity (Zhu et al. 1995). Bernauer et al. (1999, 2000) recently noted the presence of CYP2E1 in bone marrow samples of mice (several strains), rats, rabbits, and humans. However, although Irons et al. (1980) demonstrated that the isolated perfused rat femur was capable of metabolizing a very small amount of benzene (approximately 0.0002% of ^{14}C-benzene was recovered as metabolites), neither benzene oxide nor phenol were detected in a test of benzene metabolism using microsomal preparations of bone marrow from rats (Lindstrom et al. 1999), indicating that bone marrow is not a likely source of initial metabolic oxidation for benzene. No studies were located the potential for human bone marrow tissue to metabolize benzene.

Mouse liver microsomes and cytosol have been shown to catalyze ring opening in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) *in vitro*, producing *trans,trans*-muconaldehyde, a six-carbon diene dialdehyde also referred to as muconic dialdehyde (Goon et al. 1993; Latriano et al.

1986), a known hematotoxin (Witz et al. 1985) and toxic metabolite of benzene (Henderson et al. 1989). Metabolism of benzene and *trans,trans*-muconaldehyde in the isolated perfused rat liver indicated that benzene was metabolized to muconic acid, a ring-opened metabolite of benzene (Grotz et al. 1994). *Trans,trans*-muconaldehyde was metabolized to muconic acid and three other metabolites. These studies indicate that ring-opening of benzene occurs in the liver. Other recent literature identifies the following metabolites after incubation of benzene with mouse liver microsomes: phenol, hydroquinone, *trans,trans*-muconaldehyde, *6*-oxo-*trans,trans*-2,4-hexadienoic acid, 6-hydroxy-*trans,trans*-2,4-hexadienal, and 6-hydroxy-*trans,trans*-2,4-hexadienoic acid (Zhang et al. 1995a). β-Hydroxymuconaldehyde, a new metabolite, was also identified. Additional work by Zhang et al. (1995b) suggests that *cis,cis*-muconaldehyde is formed first, followed by *cis,trans*-muconaldehyde, and finally converted to *trans,trans*-muconaldehyde. Muconic dialdehyde has been shown to be metabolized *in vivo* in mice to muconic acid (Witz et al. 1990c). These data suggest that muconic dialdehyde is the precursor of muconic acid in animals exposed to benzene. Small amounts of muconic acid were found in the urine of rabbits and mice that received oral doses of ^{14}C-benzene (Gad-El-Karim et al. 1985; Parke and Williams 1953a). The percentage of this metabolite formed varied with the administered benzene dose and was quite high at low doses (17.6% of 0.5 mg/kg benzene administered to C57BL/6 mice) (Witz et al. 1990c). Other studies in animals support these results (Brondeau et al. 1992; Ducos et al. 1990; McMahon and Birnbaum 1991; Sabourin et al. 1989a; Schad et al. 1992). This pathway also appears to be active in humans (Bechtold and Henderson 1993; Ducos et al. 1990, 1992; Lee et al. 1993; Melikian et al. 1993, 1994). For instance, urine samples from male and female smokers and nonsmokers were obtained from subjects who applied for life insurance (Melikian et al. 1994). Samples from pregnant women were obtained during 7–35 weeks of pregnancy. Questionnaires were filled out on smoking history and occupation. The levels of muconic acid and cotinine (a biomarker for cigarette smoking) in the urine for the groups of pregnant and nonpregnant smokers and nonsmokers were compared with previously reported data in male smokers. Results showed the mean levels of muconic acid in the groups of male, female-nonpregnant, and female-pregnant smokers were 3.6-, 4.8-, and 4.5-fold higher than the mean concentration of this acid in the nonsmoking groups. The differences in the mean muconic acid concentrations between smoking and nonsmoking groups were significant in male, and nonpregnant and pregnant female smokers. Mean concentrations of muconic acid levels in nonpregnant female smokers are similar to that of male smokers. Mean concentrations of muconic acid in groups of 42 male smokers and 53 female smokers were 0.22±0.03 and 0.24±0.02 mg/g creatinine, or 0.13±0.06 and 0.13±0.07 mg/mg cotinine, respectively. Mean concentrations of muconic acid in groups of 63 pregnant and 53 nonpregnant female smokers were 0.27±0.04 and 0.24±0.02 mg/g creatinine, or 0.24±0.06 and 0.13±0.07 mg/mg cotinine, respectively. Because of its relative importance in benzene toxicity,

additional modeling studies, including molecular orbital studies, have been conducted to further describe how *trans,trans*-muconaldehyde is transformed to muconic acid (Bock et al. 1994).

Kenyon et al. (1995) compared their urinary profile of metabolites in B6C3F$_1$ mice after oral dosing with phenol with the results of Sabourin et al. (1989a) who administered a comparable oral dose of benzene to B6C3F$_1$ mice. The analysis of Kenyon et al. (1995) indicated that phenol administration resulted in lower urinary levels of hydroquinone glucuronide, and higher levels of phenol sulfate and phenol glucuronide compared to benzene administration. Kenyon et al. (1995) hypothesized that the differences in the urinary metabolite profiles between phenol and benzene after oral dosing were due to zonal differences in the distribution of metabolizing enzymes within the liver. Conjugating enzymes are more concentrated in the periportal area of the liver, the first region to absorb the compound, whereas oxidizing enzymes are more concentrated in the pericentral region of the liver. Based on this hypothesis, during an initial pass through the liver after oral administration, phenol would have a greater opportunity to be conjugated as it was absorbed from the gastrointestinal tract into the periportal region of the liver, thus resulting in less free phenol being delivered into the pericentral region of the liver to be oxidized. With less free phenol available for oxidation, less hydroquinone would be produced, relative to conjugated phenol metabolites. In contrast, benzene must be oxidized before it can be conjugated. Therefore, metabolism of benzene would be minimal in the periportal region of the liver, with most of the benzene reaching the pericentral region to be oxidized to hydroquinone. Based on this scheme, the authors suggest that benzene administration would result in more free phenol being delivered to oxidizing enzymes in the pericentral region of the liver than administration of phenol itself (Kenyon et al. 1995).

Benzene has been found to stimulate its own metabolism, thereby increasing the rate of toxic metabolite formation. Pretreatment of mice, rats, and rabbits subcutaneously with benzene increased benzene metabolism *in vitro* without increasing CYP2E1 concentrations (Arinc et al. 1991; Gonasun et al. 1973; Saito et al. 1973). In contrast, there was no significant effect on the metabolism of benzene when Fischer 344 rats and B6C3F$_1$ mice, pretreated with repeated inhalation exposure to 600 ppm of benzene, were again exposed to 600 ppm benzene (Sabourin et al. 1990). The rate of benzene metabolism can be altered by pretreatment with various compounds. Benzene is a preferential substrate of CYP2E1, which also metabolizes alcohol and aniline. CYP2E1 can be induced by these substrates and is associated with the generation of hydroxyl radicals, probably via futile cycling of the cytochrome (Chepiga et al. 1991; Parke 1989; Snyder et al. 1993a, 1993b). It is possible that hydroxy radical formation by CYP2E1 may play a role in the benzene ring-opening pathway, leading to the formation of *trans,trans*-muconaldehyde. Phenol, hydroquinone, benzoquinone, and catechol have also been shown to induce CYPs in human

hematopoietic stem cells (Henschler and Glatt 1995). Therefore, exposure to chemicals that stimulate the activity of this enzyme system prior to exposure to benzene could increase the rate of benzene metabolism.

Both NADPH-linked and ascorbate-induced lipid peroxidation activities induced *in vitro* were lowered 5.5 and 26%, respectively, in rats following oral administration of 1,400 mg/kg/day of benzene for 3 days, followed by intraperitoneal injection of phenobarbital. These results suggest that benzene alters hepatic drug metabolism and lipid peroxidation. The decrease in lipid peroxidation could be due to the antioxidant property of the metabolites (Pawar and Mungikar 1975).

The ultimate disposition and metabolic fate of benzene depends on animal species, dose, and route of exposure. The dose of benzene affects both the total metabolism and the concentrations of individual metabolites formed. In mice, the percentage of hydroquinone glucuronide decreased as the dose increased. In both rats and mice, the percentage of muconic acid decreased as the dose increased. The shift in metabolism may affect the dose-response relationship for toxicity, and has been observed in all animal species studies thus far (Sabourin et al. 1989a, 1992; Witz et al. 1990b, 1990c). The effect of species differences in metabolism of inhaled benzene was evidenced by the fact that mice have a higher minute volume per kg body weight than rats (1.5 times higher). This caused the blood concentration of benzene to reach equilibrium more quickly in mice than in rats, but the steady-state level in blood was not influenced (Sabourin et al. 1987). Species differences in benzene metabolism following oral exposure were elucidated in rats and mice administered benzene by gavage at doses of 0.5–150 mg/kg/day (Sabourin et al. 1987). At doses below 15 mg/kg, >90% of the benzene was metabolized, while at doses above 15 mg/kg, an increasing percentage of orally administered benzene was exhaled unmetabolized. Total metabolites per unit body weight were equal in rats and mice at doses up to 50 mg/kg/day. However, total metabolites in mice did not increase at higher doses, suggesting saturation of metabolic pathways (Sabourin et al. 1987).

The integrated dose to a tissue over a 14-hour period (6-hour exposure, 8 hours following exposure) was calculated for benzene metabolites in rats and mice that were exposed to 50 ppm of radiolabeled (^{3}H) benzene (Sabourin et al. 1988). The major metabolic products in rats were detoxification products that were marked by phenyl conjugates. In contrast, mice had substantial quantities of the markers for toxification pathways (muconic acid, hydroquinone glucuronide, and hydroquinone sulfate) in their tissues. Muconic acid and hydroquinone glucuronide were also detected in mouse bone marrow. These results may explain why mice are more susceptible to benzene-induced toxicity than rats.

In a study by Orzechowski et al. (1995), hepatocytes from adult male Wistar rats and NMRI mice were incubated for 1 hour with 0.5 mM ^{14}C-benzene, and the supernatant analyzed for metabolites. Formation of sulfate conjugates of benzene, hydroquinone, and 1,2,4-benzenetriol was also studied in a separate experiment. Mouse hepatocytes produced two metabolites (1,2,4-trihydroxybenzene sulfate and hydroquinone sulfate) that were not found in rat hepatocyte incubations. These sulfate metabolites were found in incubations including benzene, or the metabolites themselves, hydroquinone and 1,2,4-benzenetriol. Mouse hepatocytes were almost three times more effective in metabolizing benzene, compared to rat hepatocytes. This difference was accounted for in the formation of hydroquinone, hydroquinone sulfate, and 1,2,4-trihydroxybenzene sulfate. These *in vitro* experiments indicate there are both quantitative and qualitative differences in rodent metabolism of benzene.

Data produced *in vitro* by mouse and rat liver microsomes also indicate species differences in benzene metabolism (Schlosser et al. 1993). Quantitation of metabolites from the microsomal metabolism of benzene indicated that after 45 minutes, mouse liver microsomes from male B6C3F$_1$ mice had converted 20% of the benzene to phenol, 31% to hydroquinone, and 2% to catechol. In contrast, rat liver microsomes from male Fischer 344 rats converted 23% to phenol, 8% to hydroquinone, and 0.5% to catechol. Mouse liver microsomes continued to produce hydroquinone and catechol for 90 minutes, whereas rat liver microsomes had ceased production of these metabolites by 90 minutes. Muconic acid production by mouse liver microsomes was <0.04 and <0.2% from phenol and benzene, respectively, after 90 minutes.

There are quantitative differences in the benzene metabolites produced by different species (Sabourin et al. 1988). Fischer 344 rats exposed to 50 ppm benzene had undetectable amounts of phenol, catechol, and hydroquinone in the liver, lungs, and blood. The major water-soluble metabolites were muconic acid, phenyl sulfate, prephenyl mercapturic acid, and an unknown. The unknown was present in amounts equal to the amounts of phenyl sulfate in the liver; phenyl sulfate and the unknown were the major metabolites in the liver. B6C3F$_1$ mice exposed to 50 ppm benzene had detectable levels of phenol and hydroquinone in the liver, lungs, and blood; catechol was detectable only in the liver and not in the lungs or blood. As in the rat, the unknown was present in amounts equal to the amounts of phenyl sulfate in the liver. Mice had more muconic acid in the liver, which indicates a greater risk for them from *trans*,*trans*-muconaldehyde (Sabourin et al. 1988).

The effect of dose rate on benzene metabolism was studied in Fischer 344 rats and $B6C3F_1$ mice that had either long inhalation exposures to low concentrations or short exposures to high concentrations of benzene (Sabourin et al. 1989a, 1989b). Inhalation occurred at 1 of 3 exposure regimens, all having the same integral amount of benzene: 600 ppm benzene for 0.5 hour, 150 ppm for 2 hours, or 50 ppm for 6 hours. Results indicated no dose-rate effect in rats. In mice, however, the fast exposure rate (0.5 hour times 600 ppm) produced less muconic acid in the blood, liver, and lungs. In the blood and lungs, less hydroquinone glucuronide and more prephenyl mercapturic acid were produced at the higher exposure rates. At the highest benzene exposure concentrations or fastest benzene exposure rate in mice, there was a reduction in the ratios of muconic acid and hydroquinone glucuronide to the metabolite phenylsulfate. Furthermore, with increased dose rate or increased exposure concentration, mice tended to shift a greater portion of their benzene metabolism toward detoxification pathways. Likewise, the detoxification pathways for benzene appear to be low-affinity, high-capacity pathways, whereas pathways leading to the putative toxic metabolites appear to be high-affinity, low-capacity systems (Henderson et al. 1989). Accordingly, if the exposure dose regimen, via inhalation, extends beyond the range of linear metabolism rates of benzene (200 ppm by inhalation) (Sabourin et al. 1989b), then the fraction of toxic metabolites formed relative to the amount administered will be reduced. Bois and Paxman (1992) used a PBPK model to assess effects of dose rate on the disposition of benzene metabolites. Simulations were performed for rats exposed either for 15 minutes to 32 ppm or for 8 hours to 1 ppm (equivalent 8-hour TWAs). The amount of metabolites (hydroquinone, catechol, and muconaldehyde) formed was 20% higher after the 15-minute exposure at the higher level than after the 8-hour exposure at the lower level. Differences between the model predictions (Bois and Paxman 1992) and the empirical data of Sabourin et al. (1989a, 1989b) may be related, at least in part, to the higher benzene exposure levels (50, 150, and 600 ppm) used by Sabourin and coworkers.

A number of investigators have suggested that covalent binding of benzene metabolites to cellular macromolecules is related to benzene's mechanism of toxicity, although the relationship between adduct formation and toxicity is not clear. Benzene metabolites have been found to form covalent adducts with proteins from blood in humans (Bechtold et al. 1992b). Benzene metabolites form covalent adducts with nucleic acids and proteins in rats and mice (Norpoth et al. 1988; Rappaport et al. 1996); covalently bind to proteins in mouse or rat liver, bone marrow, kidney, spleen, blood, and muscle *in vivo* (Bechtold and Henderson 1993; Bechtold et al. 1992a, 1992b; Creek et al. 1997; Longacre et al. 1981a, 1981b; Sun et al. 1990); bind to proteins in perfused bone marrow preparations (Irons et al. 1980) and in rat and mouse liver DNA *in vivo* (Creek et al. 1997; Lutz and Schlatter 1977); and bind to DNA in rabbit and rat bone marrow mitochondria *in vitro* (Rushmore et al. 1984). Exposure-related increases in blood levels of

albumin adducts of benzene oxide and 1,4-benzoquinone were noted among workers occupationally exposed to benzene air concentrations ranging from 0.07 to 46.6 ppm (Rappaport et al. 2002a, 2002b). Several reactive metabolites of benzene have been proposed as agents of benzene hematotoxic and leukemogenic effects. These metabolites include benzene oxide, reactive products of the phenol pathway (catechol, hydroquinone, and 1,4-benzoquinone), and *trans,trans*-muconaldehyde. See Section 3.5.2 for a discussion of mechanisms of benzene toxicity.

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

Available human data indicate that following inhalation exposure to benzene, the major route for elimination of unmetabolized benzene is via exhalation. Absorbed benzene is also excreted in humans via metabolism to phenol and muconic acid followed by urinary excretion of conjugated derivatives (sulfates and glucuronides). In six male and female volunteers exposed to 52–62 ppm benzene for 4 hours, respiratory excretion (the amount of absorbed benzene excreted via the lungs) was approximately 17%; no gender-related differences were observed (Nomiyama and Nomiyama 1974a, 1974b). Results from a study of 23 subjects who inhaled 47–110 ppm benzene for 2–3 hours showed that 16.4–41.6% of the retained benzene was excreted by the lungs within 5–7 hours (Srbova et al. 1950). The rate of excretion of benzene was the greatest during the first hour. The study also showed that only 0.07–0.2% of the retained benzene was excreted in the urine. Other studies suggest that benzene in the urine may be a useful biomarker of occupational exposure (Ghittori et al. 1993). Results of a study involving a single human experimental subject exposed to concentrations of benzene of 6.4 and 99 ppm for 8 hours and 1 hour, respectively, suggested that excretion of benzene in breath has three phases and could possibly have four phases. The initial phase is rapid and is followed by two (or three) slower phases (Sherwood 1988). The initial phase with a high exposure concentration (99 ppm) and a short-term exposure duration (1 hour) had a more rapid excretion rate (half-life=42 minutes) and a greater percentage of the total dose excreted (17%) than did the initial phase with a low exposure concentration (6.4 ppm) and longer exposure duration (8 hours) (half-life=1.2 hours, percentage of total dose excreted=9.3%). Subsequent phases showed an increase in the half-lives. These results also showed that urinary excretion of phenol conjugate was biphasic, with an initial rapid excretion phase, followed by a slower excretion phase. A greater proportion of the total dose was excreted in urine than in breath (Sherwood 1988). The urinary excretion of phenol in workers was measured following a 7-hour workshift exposure to 1–200 ppm benzene. A correlation of 0.881 between exposure level and urinary phenol excretion was found (Inoue et al. 1986). Urine samples were collected from randomly chosen subjects not exposed to known sources of

benzene, from subjects exposed to sidestream cigarette smoke, or from supermarket workers presumed exposed to benzene from polyvinyl chloride (PVC) meat packing wrap (Bartczak et al. 1994). Samples were analyzed for identification of muconic acid. Muconic acid concentrations of 8–550 ng/mL were found in all urine samples. Kok and Ong (1994) report blood and urine levels of benzene as 110.9 and 116.4 ng/L, respectively, in nonsmokers, and 328.8 and 405.4 ng/L, respectively in smokers. A significant correlation was found between benzene levels in blood and benzene levels in urine. Similar results were found for filling station attendants in Italy (Lagorio et al. 1994b).

Popp et al. (1994) reported a mean blood benzene level in car mechanics of 3.3 μg/L. Urinary muconic acid and *S*-phenylmercapturic acid levels increased during the work shift, and were well correlated with the blood levels and the benzene air levels, which reached a maximum of 13 mg/m^3.

As discussed in Section 3.4.3, the mean urinary levels of muconic in groups of male, female-nonpregnant, and female-pregnant smokers were 3.6-, 4.8-, and 4.5-fold higher than the mean concentration of this acid in the nonsmoking groups (Melikian et al. 1994). The differences in the mean muconic acid concentrations between smoking and nonsmoking groups were significant in male (p=0.001), and nonpregnant (p=0.001) and pregnant female smokers (p=0.002). Mean concentrations of muconic acid levels in nonpregnant female smokers are similar to that of male smokers. Mean concentrations of muconic acid in groups of 42 male smokers and 53 female smokers were 0.22±0.03 and 0.24±0.02 mg/g creatinine, or 0.13±0.06 and 0.13±0.07 mg/mg cotinine, respectively. Mean concentrations of muconic acid in groups of 63 pregnant and 53 nonpregnant female smokers were 0.27±0.04 and 0.24±0.02 mg/g creatinine, or 0.24±0.06 and 0.13±0.07 mg/mg cotinine, respectively. Mean concentrations of urinary cotinine in pregnant smokers were significantly lower than in the group of nonpregnant female smokers (1.13±0.12 mg/g creatinine compared to 1.82±0.14 mg/g creatinine). Benzene levels ranging from 0.01 to 0.18 μg/kg have been detected in samples of human breast milk (Fabietti et al. 2004).

Animal data show that exhalation is the main route for excretion of unmetabolized benzene and that metabolized benzene is excreted primarily in urine. Only a small amount of an absorbed dose is eliminated in feces. A biphasic pattern of excretion of unmetabolized benzene in expired air was observed in rats exposed to 500 ppm for 6 hours, with half-times for expiration of 0.7 hour for the rapid phase and 13.1 hours for the slow phase (Rickert et al. 1979). The half-life for the slow phase of benzene elimination suggests the accumulation of benzene. The major route of excretion following a 6-hour nose-only inhalation exposure of rats and mice to various concentrations of ^{14}C-benzene appeared to be dependent on the inhaled concentration (Sabourin et al. 1987). At similar exposures to vapor

concentrations of 10–1,000 ppm, the mice received 150–200% of the equivalent dose in rats on a per kg body weight basis. At all concentrations, fecal excretion accounted for <3.5% of the radioactivity for rats and <9% for mice. At lower exposure concentrations (i.e., 13–130 ppm in rats and 11–130 ppm in mice), <6% of the radioactivity was excreted in expired air. At the highest exposure concentrations (rats, 870 ppm; mice, 990 ppm), both rats and mice exhaled a significant amount of unmetabolized benzene (48 and 14%, respectively) following termination of the exposure. The majority of the benzene-associated radioactivity that was not exhaled was found in the urine and in the carcass 56 hours after the end of exposure to these high concentrations. The radioactivity in the carcass was associated with the pelt of the animals. The authors assumed that this was due to contamination of the pelt with urine, since the inhalation exposure had been nose-only. Further investigation confirmed that the radioactivity was associated with the fur of the animals. Accordingly, the percentage of the total radioactivity excreted by these animals (urine and urine-contaminated pelt) that was not exhaled or associated with feces was 47–92% for rats and 80–94% for mice. At exposures of 260 ppm in rats, 85–92% of the radioactivity was excreted as urinary metabolites, while at exposures of 130 ppm in mice, 88–94% of the radioactivity was excreted as urinary metabolites. The total urinary metabolite formation was 5–37% higher in mice than in rats at all doses. This may be explained by the greater amount of benzene inhaled by mice per kg of body weight (Sabourin et al. 1987). Purebred Duroc-Jersey pigs were exposed to 0, 20, 100, and 500 ppm benzene vapors 6 hours/day, 5 days/week for 3 weeks (Dow 1992). The average concentration of phenol in the urine increased linearly with dose.

3.4.4.2 Oral Exposure

No studies were located regarding excretion in humans after oral exposure to benzene. Data on excretion of benzene or its metabolites in human breast milk after oral exposure were not found.

Radiolabeled benzene (340 mg/kg) was administered by oral intubation to rabbits; 43% of the label was recovered as exhaled unmetabolized benzene and 1.5% was recovered as carbon dioxide (Parke and Williams 1953a). Urinary excretion accounted for about 33% of the dose. The isolated urinary metabolites were mainly in the form of conjugated phenols. Phenol was the major metabolite accounting for about 23% of the dose or about 70% of the benzene metabolized and excreted in the urine. The other phenols excreted (percentage of dose) were hydroquinone (4.8%), catechol (2.2%), and trihydroxy-benzene (0.3%). L-Phenyl-N-acetyl cysteine accounted for 0.5% of the dose. Muconic acid accounted for 1.3%; the rest of the radioactivity (5–10%) remained in the tissues or was excreted in the feces (Parke and Williams 1953a).

Mice received a single oral dose of either 10 or 200 mg/kg radiolabeled benzene (McMahon and Birnbaum 1991). Radioactivity was monitored in urine, feces, and breath. At the low dose, urinary excretion was the major route of elimination. Hydroquinone glucuronide, phenylsulfate, and muconic acid were the major metabolites at this dose, accounting for 40, 28, and 15% of the dose, respectively. At 200 mg/kg, urinary excretion decreased to account for 42–47% of the administered dose, while respiratory excretion of volatile components increased to 46–56% of the administered dose. Fecal elimination was minor and relatively constant over both doses, accounting for 0.5–3% of the dose.

The effect of dose on the excretion of radioactivity, including benzene and metabolites, following oral administration of ^{14}C-benzene (0.5–300 mg/kg) has been studied in rats and mice (Sabourin et al. 1987). At doses of <15 mg/kg for 1 day, 90% of the administered dose was excreted in the urine of both species. There was a linear relationship for the excretion of urinary metabolites up to 15 mg/kg; above that level, there was an increased amount of ^{14}C eliminated in the expired air. Mice and rats excreted equal amounts up to 50 mg/kg; above this level, metabolism apparently became saturated in mice. In rats, 50% of the 150 mg/kg dose of ^{14}C was eliminated in the expired air; in mice, 69% of the 150 mg/kg dose of ^{14}C was eliminated in expired air (Sabourin et al. 1987). The label recovered during exhalation was largely in the form of unmetabolized benzene, suggesting that saturation of the metabolic pathways had occurred. Dose also affected the metabolite profile in the urine. At low doses, a greater fraction of the benzene was converted to putative toxic metabolites than at high doses, as reflected in urinary metabolites.

Mathews et al. (1998) reported similar results following oral (gavage) administration of ^{14}C-benzene to rats, mice, and hamsters in single doses from as low as 0.2 mg/kg and up to 100 mg/kg. For example, >95% of a 0.5 mg/kg dose was recovered in the urine of rats; a small amount (3%) was recovered in expired air. At benzene doses of 10 and 100 mg/kg, elimination in the breath rose to 9 and 50%, respectively, indicating the likely saturation of benzene metabolism. Excretion in the feces was minimal at all dose levels. Similar results were noted for mice and hamsters. Both dose and species differences were noted in the composition of urinary metabolites. Phenyl sulfate was the major metabolite in rat urine at all dose levels, accounting for 64–73% of urinary radioactivity. Phenyl sulfate (24–32%) and hydroquinone glucuronide (27–29%) were the predominant urinary metabolites in mice. At a dose of 0.1 mg/kg, mice produced a considerably higher proportion of muconic acid than rats (15 versus 7%). In hamsters, hydroquinone glucuronide (24–29%) and muconic acid (19–31%) were the primary urinary metabolites. Two additional metabolites (1,2,4-trihydroxybenzene and catechol sulfate) were recovered from the urine of hamsters, but not rats or mice.

3.4.4.3 Dermal Exposure

Limited data on excretion of benzene after dermal exposure in humans were found. Four human male subjects were given a dermal application of 0.0024 mg/cm^2 ^{14}C benzene (Franz 1984). A mean of 0.023% (range, 0.006–0.054%) of the applied radiolabel was recovered in the urine over a 36-hour period. Urinary excretion of the radiolabel was greatest in the first two hours following skin application. More than 80% of the total excretion occurred in the first 8 hours. In another study, 35–43 cm^2 of the forearm were exposed to approximately 0.06 g/cm^2 of liquid benzene for 1.25–2 hours (Hanke et al. 1961). The absorption was estimated from the amount of phenol eliminated in the urine. The absorption rate of liquid benzene by the skin (under the conditions of complete saturation) was calculated to be low, approximately 0.4 mg/cm^2/hour. The absorption due to vapors in the same experiment was negligible. Although there was a large variability in the physiological values, the amount of excreted phenol was 8.0–14.7 mg during the 24-hour period after exposure. It is estimated that approximately 30% of dermally absorbed benzene is eliminated in the form of phenol in the urine.

Data on excretion of benzene or its metabolites in human breast milk after dermal exposure were not found.

Monkeys and minipigs were exposed dermally to 0.0026–0.0036 mg/cm^2 of ^{14}C-benzene (Franz 1984). After application, the urine samples were collected over the next 2–4 days at 5-hour intervals. The rate of excretion was highest in the first two collection periods. The total urinary excretion of radioactivity was found to be higher in monkeys than in minipigs with the same exposure. Mean excretion in monkeys was 0.065% (range, 0.033–0.135%) of the applied dose compared to 0.042% (range, 0.030–0.054%) in minipigs.

Results of a study in which male rats were dermally treated with 0.004 mg/cm^2 of ^{14}C-benzene, with or without 1g of clay or sandy soil, showed that for all treatment groups, the major routes of excretion were the urine and, to a lesser extent, the expired air (Skowronski et al. 1988). The highest amount of radioactivity in urine appeared in the first 12–24 hours after treatment (58.8, 31.3, and 25.1% of the absorbed dose, respectively, for pure benzene, sandy soil–adsorbed benzene, and clay soil-adsorbed benzene). In the group treated with pure benzene, 86.2% of the absorbed dose was excreted in the urine. Sandy soil and clay soil significantly decreased urinary excretion to 64.0 and 45.4%, respectively, of the absorbed dose during the same time period. Rats receiving pure benzene excreted 12.8% of the absorbed

dose in expired air within 48 hours. Only 5.9% of the radioactivity was collected in expired air 48 hours after treatment with sandy soil–adsorbed benzene, while experiments with clay soil–adsorbed benzene revealed that 10.1% of the radioactivity was located in expired air. Less than 1% of the absorbed dose was expired as $^{14}CO_2$ in all groups. The ^{14}C activity in the feces was small (<0.5% of the applied radioactivity) in all groups 48 hours after treatment. Phenol was the major urinary metabolite detected in the 0–12-hour urine samples of all treatment groups. The percentage of total urinary radioactivity associated with phenol was 37.7% for benzene alone, 44.2% for benzene adsorbed to sandy soil, and 45.5% for benzene adsorbed to clay soil. Smaller quantities of hydroquinone, catechol, and benzenetriol were also detected (Skowronski et al. 1988).

3.4.4.4 Other Routes of Exposure

The metabolic fate of benzene can be altered in fasted animals. In nonfasted rats that received an intraperitoneal injection of 88 mg of benzene, the major metabolites present in urine were total conjugated phenols (14–19% of dose), glucuronides (3–4% of dose), and free phenol (2–3% of dose). However, in rats fasted for 24 hours preceding the same exposure, glucuronide conjugation increased markedly (18–21% of dose) (Cornish and Ryan 1965). Free phenol excretion (8–10% of dose) was also increased in fasted, benzene-treated rats. There was no apparent increase in total conjugated phenol excretion in fasted rats given benzene.

When ^{14}C-benzene (0.5 and 150 mg/kg) was injected intraperitoneally into rats and mice, most of the ^{14}C-benzene and ^{14}C-metabolites were excreted in the urine and in the expired air. A smaller amount of ^{14}C-benzene was found in the feces due to biliary excretion (Sabourin et al. 1987). Monkeys were dosed intraperitoneally with 5–500 mg/kg radiolabeled benzene, and urinary metabolites were examined (Sabourin et al. 1992). The proportion of radioactivity excreted in the urine decreased with increasing dose, whereas as the dose increased, more benzene was exhaled unchanged. This indicated saturation of benzene metabolism at higher doses. Phenyl sulfate was the major urinary metabolite. Hydroquinone conjugates and muconic acid in the urine decreased as the dose increased. When C57BL/6 mice and DBA/2 mice were given benzene subcutaneously in single doses (440, 880, or 2,200 mg/kg) for 1 day, or multiple doses (880 mg/kg) 2 times daily for 3 days, no strain differences were observed in the total amount of urinary ring-hydroxylated metabolites (Longacre et al. 1981a). Although each strain excreted phenol, catechol, and hydroquinone, differences in the relative amounts of these metabolites were noted. The more sensitive DBA/2 mice excreted more phenol but less hydroquinone than the more resistant C57BL/6 mice, while both strains excreted similar amounts of catechol. DBA/2 mice excreted more

phenyl glucuronide but less sulfate conjugate. Both strains excreted similar amounts of phenyl mercapturic acid (Longacre et al. 1981a).

3.4.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen and Krishnan 1994; Andersen et al. 1987). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parameterization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) are adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

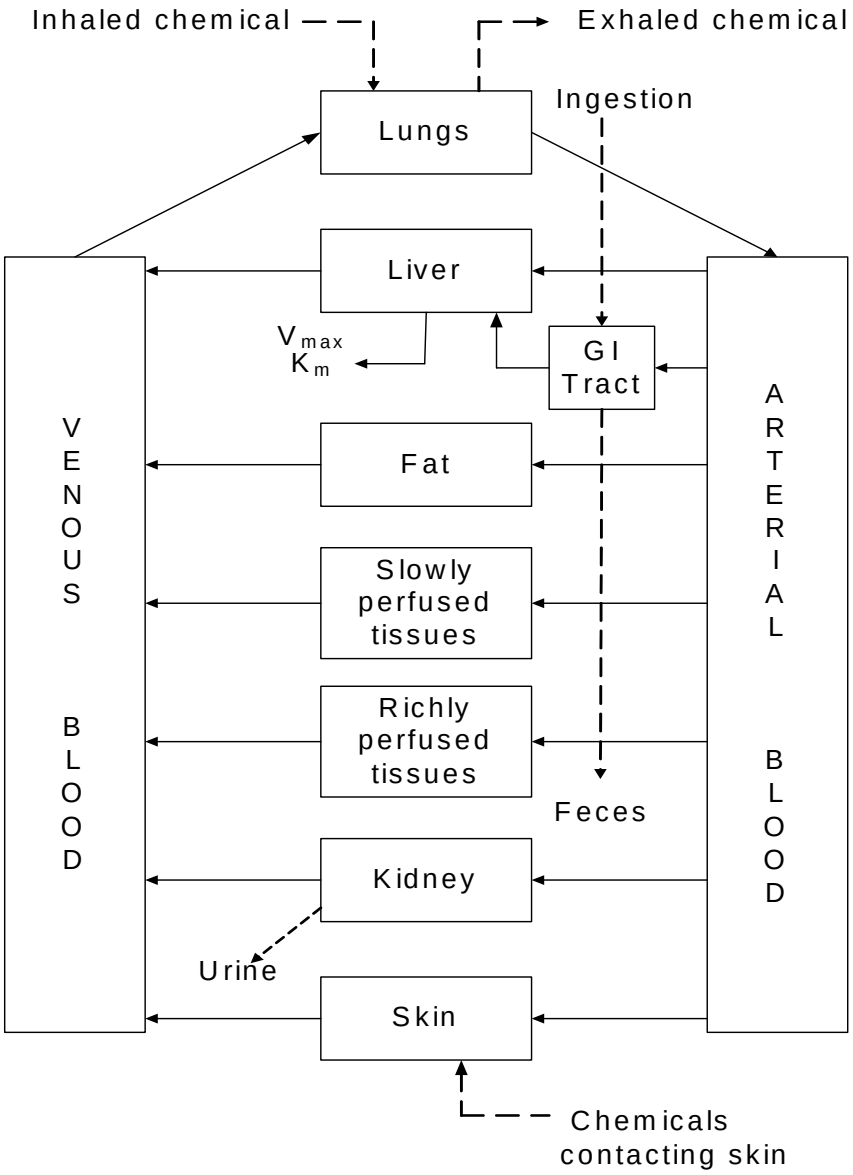
PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-4 shows a conceptualized representation of a PBPK model.

If PBPK models for benzene exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

Several PBPK models have been developed that simulate the disposition of benzene in humans (Bois et al. 1996; Brown et al. 1998; Fisher et al. 1997; Medinsky et al. 1989c; Sinclair et al. 1999; Travis et al. 1990), mice (Cole et al. 2001; Medinsky et al. 1989a, 1989b; Sun et al. 1990; Travis et al. 1990), and rats (Bois et al. 1991a; Medinsky et al. 1989a, 1989b; Sun et al. 1990; Travis et al. 1990). A comparative summary of the models is provided in Table 3-6. All of the models have the same general structure (Figure 3-5). Most of the models simulate inhalation and oral exposures; one model provides a simulation of dermal absorption (Sinclair et al. 1999). Physiological parameters and partition coefficients for simulating benzene biokinetics of human females were reported for the Brown et al. (1998) and Fisher et al. (1997) models. Flow-limited exchange of benzene between blood and tissues is assumed in all models, with excretion of benzene in exhaled air and, in one case, to breast milk (Fisher et al. 1997). All models include simulations of blood, fat, liver, lung, and lumped compartments representing other slowly-perfused tissues (e.g., skeletal muscle) and rapidly-perfused tissues (e.g., kidneys, other viscera). Simulation of bone marrow, the primary target for benzene toxicity, is included in the models reported by Bois et al. (1991a, 1996), Sinclair et al. (1999), and Travis et al. (1990).

Simulations of metabolism in the various models vary in complexity. In the simplest representation, metabolic elimination of benzene is simulated as a single capacity-limited process, represented with

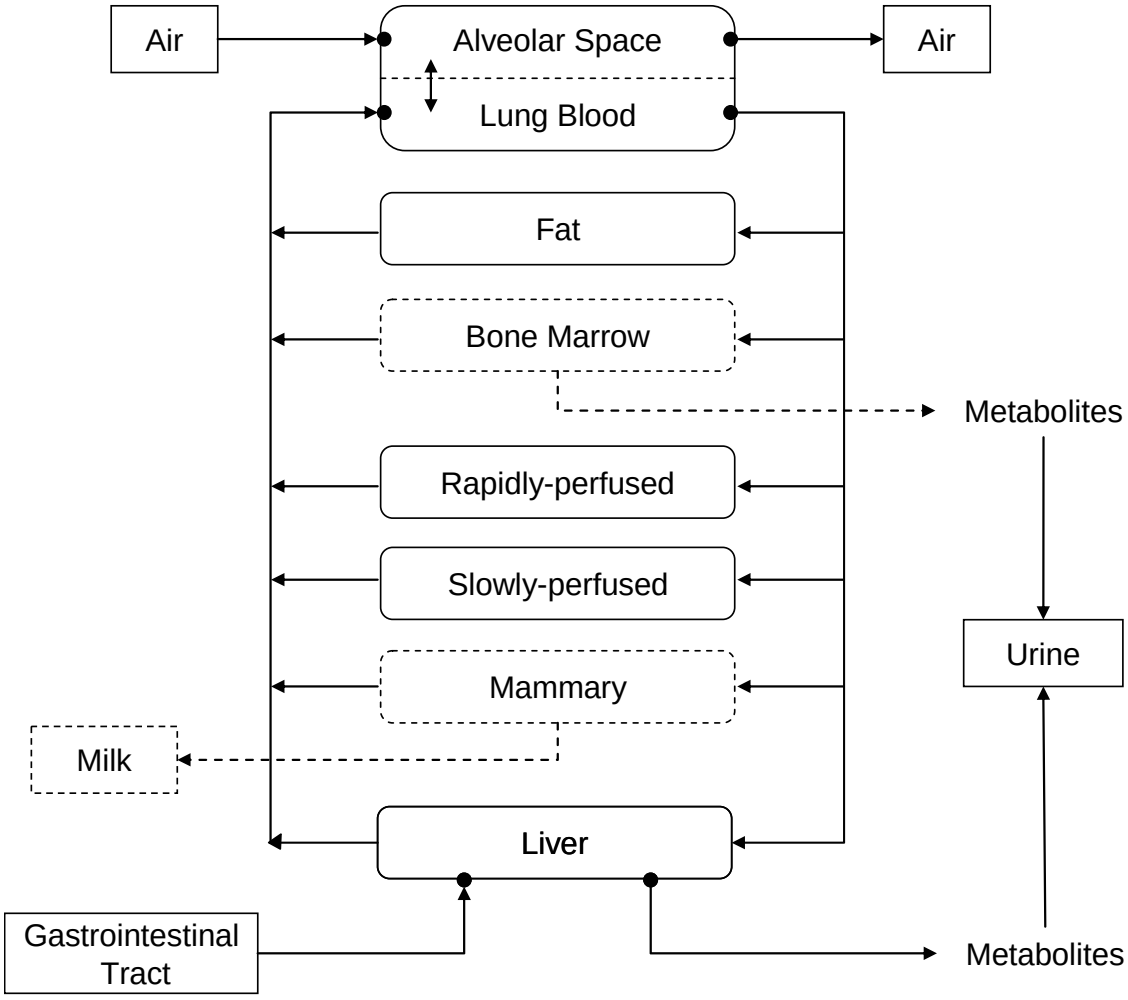
Figure 3-4. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

Source: adapted from Krishnan and Andersen 1994

Figure 3-5. General Structure of Physiologically Based Pharmacokinetic Models of Benzene*



*Tissues shown with dashed lines are not simulated in all models. Flow-limited exchange of benzene between blood and tissues is assumed. Metabolism is simulated to varying degrees of complexity (see Table 3-6 for model comparison).

Table 3-6. Summary Comparison of Physiologically Based Pharmacokinetic Models for Benzene

Reference	Simulated areas: Species[a]	Absorption pathways[b]	Tissues[c]	Metabolic pathways[d]		Excretion pathways[e]		Comment
Bois et al. 1991a	R	IH, OR	BL, BM, FA, LI, LU, RP, SP	BM, LI:	BZ→BO(c)	EH:	BZ	Simulates metabolic pathways in bone marrow, and phenol conjugation in lung and gastrointestinal tract
					BO→BG(c)	UR:	PH	
					BO→PH(f)			
					BO→GSH(c)			
					BG→DI(c)			
					PH→HQ(c)			
					PH→CA(c)			
				BM, LU, LU, GI:	PH→PHCO(c)			
Bois et al. 1996	H	IH	BL, BM, FA, LU, LI, RP, SP	BM, LI:	BZ→M_{tot}(c)	EH:	BZ	Simulates metabolic pathways in bone marrow, and endogenous production of phenolic metabolites
				LI:	PHX_{end}→PH(z)	UR:	M_{tot}	
							PH	
Brown et al. 1998	H (m,f)	IH	BL, FA, LI, LU, RP, SP	LI:	BZ→M_{tot}(f)	EH:	BZ	Simulates males or females
Cole et al. 2001	M	IH, OR	BL, FA, LI, LU, RP, SP	LI:	BZ→BO(c)	EH:	BZ	All metabolism is assigned to the liver
					BO→PH(f)	UR:	CA	
					BO→PMA(f)		MA	
					BO→MA(f)		PHCO	
					PH→HQ(c)		PMA	
					PH→PHCO(c)		HQCO	
					PH→CA(c)		THB	
					CA→THB(c)			
					HQ→HQCO(c)			
Fisher et al. 1997	H	IH	FA, LU, LI, RP, SP, MI	LI:	BZ→M_{tot}(c)	EH:	BZ	Simulates transfer of benzene to breast milk
						MI:	BZ	
Medinsky et al. 1989a, 1989b, 1989c	H, M, R	IH, OR	FA, LI, LU, RP, SP	LI:	BZ→BO(c)	EH:	BZ	All metabolism is assigned to the liver
					BO→PHCO(c)			
					BO→PMA(c)			
					BO→HQCO(c)			
					BO→MA(c)			

Table 3-6. Summary Comparison of Physiologically Based Pharmacokinetic Models for Benzene

Reference	Simulated areas							Comment
	Species[a]	Absorption pathways[b]	Tissues[c]	Metabolic pathways[d]		Excretion pathways[e]		
Sinclair et al. 1999	H	IH, OR, DE	BL, BM, LI, LU, MU, RP	BM, LI:	$BZ \rightarrow M_{tot}(c)$	EH:	BZ	Simulates dermal exposure and absorption
						UR:	M_{tot}	
							PH	
Sun et al. 1990	M, R	IH, OR	BL, FA, LI, LU, RBC, RP, SP	LI:	$BZ \rightarrow BO(c)$	EH:	BZ	Simulates formation of hemoglobin adducts in red blood cells derived from benzene oxide
					$BO \rightarrow PHCO(c)$			
					$BO \rightarrow PMA(c)$			
					$BO \rightarrow HQCO(c)$			
					$BO \rightarrow MA(c)$			
				RBC:	$BO \rightarrow HBA(c,f)$			
Travis et al. 1990	H, M, R	IH, OR	BL, BM, FA, LI, LU, MU, RP	BM, LI:	$BZ \rightarrow M_{tot}(c)$	EH:	BZ	Total metabolism of benzene in the bone marrow and liver

[a]Species simulated: H=human; M=mouse; R=rat; m=male; f=female
[b]Absorption pathways simulated: IH=inhalation; OR=oral; DE=dermal
[c]Tissues simulated: BL=blood; BM=bone marrow; FA=fat; LI=liver; LU=lung; MU=muscle; RBC=red blood cells; RP=other rapidly-perfused tissues; SP=other slowly-perfused tissues
[d]Metabolic pathways simulated: BZ=benzene; BD=benzene diols; BO=benzene oxide; BG=Benzene glycol; CA=catechol; HBA=hemoglobin adduct; HQ=hydroquinone; HQCO=ydroqinione conjugates; MA=muconic acid; M_{tot}=total metabolites; PH=phenol; PHCO=phenol conjugates; PMA=phenylmercapturic acid; THB=trihydroxybenzene; PHX_{end}=endogenous phenolic metabolites; (c)=capacity-limited; (f)=first-order; (z)= zero-order
[e]Excretion pathways simulated: EH=exhalation; MI=breast milk; UR=urine

Michaelis-Menten function of benzene concentration in tissue (Bois et al. 1996; Brown et al. 1998; Fisher et al. 1997; Sinclair et al. 1999; Travis et al. 1990). In the more complex representations, the major pathways of metabolism of benzene, including conjugation reactions, are simulated as capacity-limited or first-order processes (Bois et al. 1991a; Cole et al. 2001; Medinsky et al. 1989a, 1989b, 1989c; Sun et al. 1990). In most of the models, all metabolic pathways are attributed to the liver; however, four of the models include simulations of metabolism in bone marrow (Bois et al. 1991a, 1996; Sinclair et al. 1999; Travis et al. 1990), and one model includes simulations of the formation of sulfate and glucuronide conjugates of phenol in the gastrointestinal and respiratory tracts (Bois et al. 1991a). The Sun et al. (1990) model includes a simulation of the formation of hemoglobin adducts derived from benzene oxide. In models that simulate the disposition of the metabolites, metabolites are assumed to be excreted in urine either at a rate equal to their formation (Cole et al. 2001), or in accordance with a first-order excretion rate constant (Bois et al. 1991a, 1996; Sinclair et al. 1999); the difference being, in the latter, the mass balance for formation and excretion of metabolites is simulated, allowing predictions of metabolite levels in tissues. All of the models use typical parameters and values for species-specific blood flows and tissue volumes.

Brief summaries of the models presented in Table 3-6 are provided below, with emphasis on unique features that are applicable to risk assessment.

Medinsky et al. 1989a, 1989b, 1989c

Description of the Model. The Medinsky et al. (1989a, 1989b, 1989c) model simulates absorption and disposition of benzene in the human, mouse, and rat. Tissues simulated include the blood, bone marrow, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Gastrointestinal absorption of benzene is simulated as a first-order process; absorption and excretion of benzene in the lung are assumed to be flow-limited. Exchange of benzene between blood and tissues is assumed to be flow-limited. The model simulates capacity-limited (i.e., Michaelis-Menten) metabolism of benzene to benzene oxide as a function of the concentration of benzene in liver. Conversion of benzene oxide to phenol conjugates, phenylmercapturic acid, hydroquinone conjugates, and muconic acid are simulated as parallel, capacity-limited reactions in liver. The model simulates rates of formation of metabolites, but not the disposition (e.g., excretion) of metabolites. Metabolism parameter values (V_{max}, K_m) for the mouse and rat models were estimated by optimization of the model to observations of total metabolites formed in mice and rats exposed by inhalation or oral routes to benzene (Medinsky et al. 1989b; Sabourin

et al. 1987). Human metabolism parameter values were derived from allometric scaling of the values for mice (Medinsky et al. 1989c).

Risk Assessment. The model has been used to predict the amounts of benzene metabolites formed in rats and mice after inhalation or oral exposures (Medinsky et al. 1989a, 1989b). For inhalation concentrations up to 1,000 ppm, mice were predicted to metabolize at least 2–3 times more benzene than rats. For oral doses >50 mg/kg, rats were predicted to metabolize more benzene on a kg-body weight basis than mice. The model also predicts different metabolite profiles in the two species: mice were predicted to produce primarily hydroquinone glucuronide and muconic acid, metabolites linked to toxic effects, whereas rats were predicted to produce primarily phenyl sulfate, a detoxification product. These predictions agree with experimental data and provide a framework for understanding the greater sensitivity of the mouse to benzene toxicity.

Validation of the Model. The model was calibrated with data from Sabourin et al. (1987). Bois et al. (1991b) compared predictions made to observations of benzene exhaled by rats following exposures to 490 ppm benzene, reported by Rickert et al. (1979), as well as the data from which the model was calibrated (Sabourin et al. 1987). In general, the model tended to overestimate observations to which it was not specifically fitted.

Target Tissues. The model simulates amounts and concentrations of benzene in blood, liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues as well as amounts of metabolites formed. It does not simulate concentrations of metabolites in these tissues. It does not simulate bone marrow, a target of benzene metabolites.

Species Extrapolation. The model has been applied to simulations of mice, rats, and humans (Medinsky et al. 1989a, 1989b, 1989c).

High-low Dose Extrapolation. The model has been evaluated for simulating inhalation exposures in rodents ranging from 1 to 1,000 ppm and oral gavage doses ranging from 0.1 to 300 mg/kg (Bois et al. 1991b; Medinsky et al. 1989a, 1989b, 1989c).

Interroute Extrapolation. The model simulates inhalation and oral exposures and has been applied to predicting internal dose metrics (e.g., amounts of metabolites formed) resulting from exposures by these routes (Medinsky et al. 1989a, 1989b, 1989c).

Strengths and Limitations. Strengths of the model are that it simulates disposition of inhaled and ingested (single dose) benzene, including rates and amounts of major metabolites formed in mice, rats, and humans. Limitations include: (1) the model has not been evaluated for multiple exposures; (2) the model attributes all metabolism to the liver; (3) the model does not simulate the fate of metabolites formed and, therefore, cannot be used to predict concentrations of metabolites (e.g., muconaldehyde) in tissues; and (4) the model does not simulate bone marrow, a major target tissue for benzene metabolites.

Sun et al. 1990

Description of the Model. The Sun et al. (1990) model is an extension of the mouse and rat models developed by Medinsky et al. (1989a, 1989b, 1989c). The Sun et al. (1990) model includes a simulation of the formation of hemoglobin adducts derived from benzene oxide. Adduct formation is represented as the sum of capacity-limited and first-order functions of the concentration of benzene oxide in the liver. Parameter values were estimated by optimization to measurements of hemoglobin adduct formations in rats and mice exposed to single oral gavage doses of benzene (Sun et al. 1990).

Risk Assessment. The model has been applied to predicting the levels of hemoglobin adducts in mice and rats following inhalation or oral exposures to benzene. This approach could be potentially useful for predicting exposure levels that correspond to measured hemoglobin adduct levels, for use of adducts as an exposure biomarker.

Validation of the Model. The model was calibrated against measurements of hemoglobin adduct formation in mice and rats that received single oral gavage doses of benzene ranging from 0.008 to 800 mg/kg (Sun et al. 1990). The model was evaluated by comparing predictions to observations of amounts of hemoglobin adducts formed in mice and rats exposed to benzene vapor concentrations of 5, 50, or 600 ppm for 6 hours (Sabourin et al. 1989a).

Target Tissues. The model predicts hemoglobin adduct formation after oral and inhalation exposure (Sun et al. 1990).

Species Extrapolation. The model has been applied to simulations for mice and rats.

High-low Dose Extrapolation. The model was calibrated with observations made in mice and rats exposed to single gavage doses ranging from 0.1 to 10,000 μmol/kg (0.008–800 mg/kg), and evaluated for predicting observations in mice and rats exposed by inhalation to 600 ppm benzene.

Interroute Extrapolation. The Sun model examined two routes of exposure, oral and inhalation. The model was found to be useful in predicting the concentrations of hemoglobin adducts in blood in rodents after oral and inhalation exposure.

Strengths and Limitations. Strengths of the model are that it extends the Medinsky et al. (1989a, 1989b, 1989c) models to simulate hemoglobin adduct formation secondary to formation of benzene oxide. A limitation of the adduct model is that it simulates production of adducts as a function of benzene oxide concentration in liver and does not consider other potential pathways of adduct formation through hydroquinone, phenol, or muconaldehyde.

Travis et al. 1990

Description of the Model. The Travis et al. (1990) model simulates the absorption and disposition of benzene in the human, mouse, and rat. Tissues simulated include the blood, bone marrow, fat, liver, lung, other slowly-perfused tissues, and rapidly-perfused tissues. Gastrointestinal absorption of benzene is simulated as a first-order process. Absorption and excretion of benzene in the lung are assumed to be flow-limited, as are exchanges of benzene between blood and tissues. The model simulates capacity-limited (i.e., Michaelis-Menten) metabolic elimination of benzene as a function of the concentration of benzene in bone marrow and liver. The model simulates rates of metabolic elimination of benzene, but not the rates of formation of specific metabolites or their disposition (e.g., excretion). For the purpose of comparing model predictions to observations, 80% of the total metabolite formed in 24 hours (and excreted in urine) was assumed to be phenol. Metabolism parameter values (V_{max}, K_m) were estimated by optimization of the model to observations of total metabolites formed (i.e., excreted in urine) in humans, mice, and rats exposed to benzene by inhalation or oral routes to benzene. The V_{max} for metabolism in bone marrow in humans was assumed to be 4% of that of liver, consistent with optimized values for rodents.

Risk Assessment. This model has been used to predict the amounts of benzene in expired air, concentrations of benzene in blood, and total amount of benzene metabolized following inhalation exposures to humans and inhalation, intraperitoneal, oral gavage, or subcutaneous exposures in mice or

rats (Travis et al. 1990). Cox (1996) applied the model to derive internal dose-response relationships for benzene in humans.

Validation of the Model. The model was evaluated by comparing predictions with observations made in mouse and rat inhalation studies (Rickert et al. 1979; Sabourin et al. 1987; Sato et al. 1975; Snyder et al. 1981); mouse oral gavage studies (Sabourin et al. 1987); mouse subcutaneous injection studies (Andrews et al. 1977); and rat intraperitoneal injection studies (Sato and Nakajima 1979). Predictions of benzene in expired air and/or blood concentrations were also compared to observations made in humans who inhaled concentrations ranging from 5 to 100 ppm benzene (5 ppm: Berlin et al. 1980; Sherwood 1972; 25–57 ppm: Sato et al. 1975; Sherwood 1972; Nomiyama and Nomiyama 1974a, 1974b; 99–100 ppm: Sherwood 1972; Teisinger and Fiserova-Bergerova 1955). Further evaluations of predictions of benzene in workers are reported in Sinclair et al. (1999) and Sherwood and Sinclair (1999), who compared model predictions with observations of benzene in exhaled breath and urinary excretion of phenol in workers who were exposed to benzene at concentrations ranging from 1 to 1,100 ppm.

Target Tissues. The model simulates amounts and concentrations of benzene in blood, bone marrow (a target tissue), liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues; and amounts of metabolites formed in liver and bone marrow. It does not simulate concentrations of metabolites in these tissues.

Species Extrapolation. The model has been applied to simulations for mice, rats, and humans (Sherwood and Sinclair 1999; Sinclair et al. 1999; Travis et al. 1990).

High-low Dose Extrapolation. The model has been evaluated for simulating inhalation exposures in humans ranging from 1 to 1,110 ppm (Sherwood and Sinclair 1999; Sinclair et al. 1999; Travis et al. 1990). Evaluations of predictions in rodents included observations made during inhalation exposures that ranged from 11 to 1,000 ppm and oral gavage doses that ranged from 0.5 to 300 mg/kg.

Interroute Extrapolation. The model simulates inhalation and oral exposures and has been applied to predicting internal dose metrics (e.g., benzene concentration in blood, amount benzene metabolized) resulting from exposures by these routes (Travis et al. 1990).

Strengths and Limitations. Strengths of the model are that it simulates (1) disposition of inhaled and ingested (single dose) benzene in mice, rats, and humans; and (2) concentrations of benzene, and

rates and amount of benzene metabolized in bone marrow, a target tissue for benzene metabolites. Limitations of the model include: (1) the model simulates metabolic elimination of benzene, but not the rates of formation of major metabolites; and (2) the model does not simulate fate of metabolites formed and, therefore, cannot be used to predict concentrations of metabolites in tissues.

Fisher et al. 1997

Description of the Model. The Fisher et al. (1997) model extends the model reported by Travis et al. (1990) to include a simulation of lactational transfer of benzene to breast milk in humans. Other tissues simulated include blood, fat, liver, lung, other slowly-perfused tissues, and rapidly-perfused tissues. Absorption and excretion of benzene in the lung and exchange of benzene between blood and tissues are assumed to be flow-limited, as is excretion of benzene in breast milk. The lactational transfer model includes simulations of breast milk production and loss from nursing; the latter is represented as a first-order process. Estimates of blood:air and blood:milk partition coefficients during lactation (from which the milk:blood partition coefficient could be calculated) were measured in nine lactating subjects (Fisher et al. 1997). The model simulates capacity-limited (i.e., Michaelis-Menten) metabolism of metabolic elimination of benzene as a function of the concentration of benzene in liver. Rates of formation of specific metabolites and their disposition (e.g., excretion) are not simulated. Metabolism parameter values (K_m,V_{max}) and tissue:blood partition coefficients were derived from Travis et al. (1990).

Risk Assessment. This model has been used to predict benzene concentrations in breast milk and lactational transfers to breast feeding infants (Fisher et al. 1997). Exposures to the threshold limit value (TLV) (10 ppm, 8 hours/day, 5 days/week) were predicted to yield 0.053 mg of benzene in breast milk per 24 hours. This approach has potential applicability to assessing lactational exposures to infants resulting from maternal exposures.

Validation of the Model. The lactation model was evaluated (Fisher et al. 1997) by comparing predictions for perchloroethylene (not benzene) with those predicted by a perchloroethylene model developed by Schreiber (1993). Other components of the biokinetics model were derived from the Travis et al. (1990) model, which has undergone evaluations against data obtained from studies in humans.

Target Tissues. The model simulates concentrations of benzene in blood, breast milk, liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues as well as rates of metabolic

elimination of benzene. It does not simulate concentrations of metabolites in these tissues and does not simulate metabolism in bone marrow, a major target of benzene metabolites.

Species Extrapolation. The model has been applied to simulations for humans (Fisher et al. 1997).

High-low Dose Extrapolation. The lactational model has not been evaluated for simulating inhalation exposures to benzene in humans; therefore, applicability to high-low dose extrapolations cannot be assessed.

Interroute Extrapolation. The model was developed to simulate inhalation exposures. Extrapolation to other routes (e.g., oral, dermal) would require the extension of the model to include simulations of absorption from these routes.

Strengths and Limitations. Strengths of the model are that it simulates the disposition of inhaled benzene in females during lactation, including transfers of benzene to breast milk and nursing infants; concentrations of benzene in blood and tissues; and rates of metabolic elimination of benzene metabolized. Limitations of the model include that the model does not simulate rates of formation of major metabolites and that the model does not simulate kinetics of uptake or metabolism of benzene in bone marrow, a major target of benzene toxicity.

Sinclair et al. 1999

Description of the Model. The Sinclair et al. (1999) model is an extension of the human model developed by Travis et al. (1990) to include a simulation of first-order urinary excretion of total metabolites and phenol, and dermal absorption of benzene.

Risk Assessment. The model has been applied to predicting the levels of benzene in exhaled air and phenol in urine in workers exposed to benzene (Sherwood and Sinclair 1999; Sinclair et al. 1999).

Validation of the Model. The model was evaluated against measurements of benzene in exhaled breath and urinary excretion of phenol in workers who were exposed to benzene at concentrations ranging from 1 to 1,100 ppm (Sherwood and Sinclair 1999; Sinclair et al. 1999).

Target Tissues. The model simulates amounts and concentrations of benzene in blood, bone marrow (a target tissue), liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues; rates of metabolic elimination of benzene in liver and bone marrow; and excretion of total metabolites formed and phenol.

Species Extrapolation. The model has been applied to simulations for humans (Sinclair et al. 1999).

High-low Dose Extrapolation. The model was evaluated against observations of benzene in exhaled breath and urinary excretion of phenol in workers who were exposed to benzene at concentrations ranging from 1 to 1,100 ppm (Sherwood and Sinclair 1999; Sinclair et al. 1999).

Interroute Extrapolation. The model simulates inhalation, oral, and dermal exposures.

Strengths and Limitations. Strengths of the model are that it extends the Travis et al. (1990) model to include simulation of dermal absorption of benzene.

Bois et al. 1991a

Description of the Model. The Bois et al. (1991a) model simulates absorption and disposition of benzene and the benzene metabolite, phenol, in the rat. Tissues simulated include the blood, bone marrow, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Gastrointestinal absorption of benzene and phenol are simulated as a first-order function for dose. Absorption and excretion of benzene in the lung are assumed to be flow-limited as are exchanges of benzene and phenol between blood and tissues. Excretion of phenol is simulated as a first-order transfer to urine. The model simulates capacity-limited (i.e., Michaelis-Menten) and first-order metabolism of benzene and metabolites in bone marrow, liver, gastrointestinal tract, and respiratory tract (see Table 3-6). All pathways are assumed to be capacity-limited reactions, except for the spontaneous hydrolysis of benzene oxide to form phenol, which is simulated as a first-order process. The model simulates rates of formation of metabolites and first-order excretion of phenol; however, disposition (e.g., excretion) of other metabolites is not simulated. Parameter values, including metabolism parameter values, were optimized to a reference set of observations of metabolites formed in rats exposed by inhalation or to single gavage doses of benzene (see below).

Risk Assessment. This model has been used to predict amounts of benzene and phenol metabolites formed in rats during oral gavage exposures to benzene equivalent to those administered in NTP (1986) and to inhalation exposures equivalent to the OSHA PEL (Bois and Paxman 1992; Bois et al. 1991a). Model simulations indicate that dose rate may be an important factor in benzene toxicity. For example, when the model was applied to simulations for rats exposed either for 15 minutes to a benzene vapor concentration of 32 ppm or for 8 hours to 1 ppm (equivalent 8-hour TWAs), the amount of metabolites (hydroquinone, catechol, and muconaldehyde) formed was 20% higher after the 15-minute exposure at the higher level than after the 8-hour exposure at the lower level (Bois and Paxman 1992). These metabolites have been identified as being important in the genesis of bone marrow toxicity after benzene exposure (Eastmond et al. 1987). These types of analyses, if extended to humans, would be applicable to evaluations of the adequacy of short-term exposure limits.

Validation of the Model. The model was calibrated (Bois and Paxman 1992; Bois et al. 1991a) with observations made in rats exposed to single oral gavage doses of benzene, or to inhalation exposures of 13–870 ppm (Sabourin et al. 1987, 1989b), in rats administered single parenteral doses of phenol (Cassidy and Houston 1984), and in *in vitro* metabolism studies (Sawahata and Neal 1983). Further evaluations against data not used in the calibration were not reported.

Target Tissues. The model simulates amounts and concentrations of benzene and phenol in bone marrow, a target tissue for benzene metabolites, as well as in blood, liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues. The model also simulates amounts of specific metabolites formed and urinary excretion of the major urinary metabolite, phenol. It does not simulate concentrations of metabolites, other than phenol, in these tissues.

Species Extrapolation. The model has been applied to simulations for rats. A human model has been developed that implements a scaled-down version of the rat metabolism model (see Bois et al. 1996).

High-low Dose Extrapolation. The model has been evaluated for simulating inhalation exposures in rats ranging from 13 to 870 ppm and oral gavage doses ranging from 15 to 300 mg/kg.

Interroute Extrapolation. The model simulates inhalation and oral exposures and has been applied to predicting internal dose metrics (e.g., amounts of metabolites formed) resulting from exposures by these routes.

Strengths and Limitations. Strengths of the model are that it simulates disposition of inhaled and ingested benzene (and phenol), including rates and amounts of most of the major metabolites formed in rats. Limitations include: (1) the model has not been evaluated for multiple exposures; (2) although the model simulates the fate of benzene and phenol, it does not simulate the fate of other metabolites formed and, therefore, cannot be used to predict concentrations of these metabolites in tissues; and (3) the model, as configured in Bois et al. (1991a), does not simulate benzene disposition in humans.

Bois et al. 1996

Description of the Model. The Bois et al. (1996) model simulates inhalation absorption and disposition of benzene in humans. Tissues simulated include the blood, bone marrow, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Absorption and excretion of benzene in the lung are assumed to be flow-limited as are exchanges of benzene between blood and tissues. The model simulates metabolic elimination of benzene as a single capacity-limited (i.e., Michaelis-Menten) reaction, occurring in bone marrow and liver. Endogenous formation of phenolic metabolites is also simulated as a zero-order process occurring in liver. The model simulates first-order excretion of total metabolites and the phenol fraction (approximately 80% of total). Parameter values (physiological and chemical) were estimated by Bayesian optimization techniques (Markov Chain Monte Carlo analysis) using reference observations of benzene concentration in blood and urinary excretion of phenol in human subjects who were exposed to benzene in air (Pekari et al. 1992).

Risk Assessment. The model has been used to predict rates and amounts of benzene metabolized in human populations (Bois et al. 1996). The population model (population geometric means and standard deviations of parameter values) was derived using Markov Chain Monte Carlo analysis with observations from three human subjects serving as the reference data for inter-individual variability (from Pekari et al. 1992). The population model predicts probability distributions of model outputs (for example, rates or amounts of benzene metabolized for a given exposure). This approach could be used to evaluate uncertainty factors in risk assessments intended to account for uncertainties in our understanding of benzene pharmacokinetics variability.

Validation of the Model. The model was calibrated with observations of benzene concentrations in blood and urinary phenol levels, made in three human subjects who were exposed to 1.7 or 10 ppm

benzene for 4 hours (Pekari et al. 1992). Further evaluations against data not used in the calibration have not been reported.

Target Tissues. The model simulates amounts and concentrations of benzene in bone marrow, a target of benzene toxicity, as well as blood, liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues. Amounts of total metabolites formed and excreted are simulated; however, the model does not simulate concentrations of metabolites in these tissues.

Species Extrapolation. The model has been applied to simulations for humans (Bois et al. 1996).

High-low Dose Extrapolation. The model has been evaluated for simulating inhalation exposures in human subjects ranging from 1.7 to 10 ppm (Bois et al. 1996).

Interroute Extrapolation. The model simulates inhalation exposures. Extrapolation to other routes (e.g., oral, dermal) would require the extension of the model to include simulations of absorption from these routes.

Strengths and Limitations. Strengths of the model are that it simulates disposition of inhaled benzene and rates of total metabolism in humans. Limitations include that the model has not been evaluated for multiple exposures and that the model simulates total metabolism of benzene, and not the rates of formation of the major metabolites of benzene of toxicological interest.

Brown et al. 1998

Description of the Model. The Brown et al. (1998) model simulates inhalation absorption and disposition of benzene in humans. Tissues simulated include the blood, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Absorption and excretion of benzene in the lung and exchange of benzene between blood and tissues are assumed to be flow-limited. The model simulates capacity-limited (i.e., Michaelis-Menten) metabolic elimination of benzene as a function of the concentration of benzene in liver. Rates of formation of specific metabolites, or their disposition (e.g., excretion), are not simulated. For the purpose of comparing model predictions to observations, 80% of the total metabolites formed and excreted in urine (i.e., amount of benzene eliminated by metabolism) in 24 hours was assumed to be phenol. The K_m parameter for metabolism was derived from Travis et al. 1990; the V_{max} was estimated by optimization of the model to observations of blood concentrations of

benzene and benzene in exhaled breath of female and male subjects who were exposed to 25 ppm benzene for 2 hours (Sato et al. 1975). Partition coefficients for males and females were derived from vial equilibrium studies conducted on blood and/or tissues from males and females (Fisher et al. 1997; Paterson and Mackay 1989).

Risk Assessment. This model has been used to predict the benzene concentrations in blood and amounts of benzene metabolized in females and males who experience the same inhalation exposure scenarios. Females were predicted to metabolize 23–26% more benzene than similarly-exposed males. This difference was attributed, in part, to a higher blood:air partition coefficient for benzene in females.

Validation of the Model. The model was calibrated by comparing predictions of blood concentrations of benzene and benzene in exhaled breath of female and male subjects who were exposed to 25 ppm benzene for 2 hours (Brown et al. 1998; Sato et al. 1975). Further evaluations against data not used in the calibration were not been reported.

Target Tissues. The model simulates concentrations of benzene in blood, liver, fat, and lumped compartments for other rapidly-perfused and slowly-perfused tissues as well as rates of metabolic elimination of benzene. It does not simulate concentrations of metabolites in these tissues and does not simulate metabolism in bone marrow, a major target of benzene metabolites.

Species Extrapolation. The model has been applied to simulations for humans.

High-low Dose Extrapolation. The model has been evaluated for simulating inhalation exposures in humans. Evaluations of predictions included observations made during inhalation exposures to 25 ppm (Brown et al. 1998; Sato et al. 1975).

Interroute Extrapolation. The model simulates inhalation and has been applied to predicting internal dose metrics (e.g., benzene concentration in blood, amount benzene metabolized) resulting from exposures by this route (Brown et al. 1998). Extrapolation to other routes (e.g., oral, dermal) would require the extension of the model to include simulations of absorption from these routes.

Strengths and Limitations. Strengths of the model are that it simulates disposition of inhaled benzene in female and male humans as well as the concentrations of benzene and rates of metabolic elimination of benzene metabolized. Limitations of the model include: (1) the model does not simulate

rates of formation of major benzene metabolites; (2) the model does not simulate fate of metabolites formed and, therefore, cannot be used to predict concentrations of metabolites in tissues; and (3) the model does not simulate kinetics of uptake or metabolism of benzene in bone marrow, a major target of benzene toxicity.

Cole et al. 2001

Description of the Model. The Cole et al. (2001) model simulates absorption and disposition of benzene in the mouse. Tissues simulated include the blood, fat, liver, lung, other slowly-perfused tissues, and other rapidly-perfused tissues. Gastrointestinal absorption of benzene is simulated as a first-order process. Absorption and excretion of benzene in the lung are assumed to be flow-limited as are exchanges of benzene between blood and tissues. The model simulates capacity-limited (i.e., Michaelis-Menten) and first-order metabolism of benzene and metabolites in liver (see Table 3-6). Capacity-limited reactions in bone marrow and liver include benzene to benzene oxide, phenol to hydroquinone, phenol to catechol, catechol to trihydroxybenzene, and conjugation of phenol and hydroquinone. First-order reactions in liver include conversion of benzene oxide to phenol, muconic acid, and phenylmercapturic acid. The model simulates rates of formation of metabolites, tissue distribution of benzene oxide, phenol, and hydroquinone; and first-order excretion of metabolites in urine. Capacity-limited metabolism parameter values were estimated from *in vitro* studies of mouse liver (Lovern et al. 1999; Nedelcheva et al. 1999; Seaton et al. 1995); first-order parameters were estimated by optimization of model output to observations of metabolites formed in mice exposed by inhalation or to single gavage doses (Kenyon et al. 1995; Mathews et al. 1998; Sabourin et al. 1988). Blood:tissue partition coefficients for benzene and metabolites were derived from Medinsky et al. (1989a) or estimated based on the n-octanol-water partition coefficient (Poulin and Krishnan 1995).

Risk Assessment. This model has been used to predict amounts of benzene exhaled and amounts of benzene metabolites produced in mice during inhalation exposures or following oral gavage exposures to benzene (Cole et al. 2001).

Validation of the Model. The model was calibrated with observations made in mice exposed to single oral gavage doses of benzene, or to inhalation exposures (Cole et al. 2001; Kenyon et al. 1995; Mathews et al. 1998; Sabourin et al. 1988). Further evaluations against data not used in the calibration have not been reported.

Target Tissues. The model simulates amounts and concentrations of benzene in blood, liver, fat, and lumped compartments for other rapidly perfused and slowly perfused tissues; rates of formation of metabolites; tissue distribution of benzene oxide, phenol, and hydroquinone; and first-order excretion of metabolites in urine. It does not simulate concentrations of metabolites in bone marrow, a target tissue for benzene metabolites.

Species Extrapolation. The model has been applied to simulations for mice (Cole et al. 2001).

High-low Dose Extrapolation. The model has been evaluated for simulating inhalation exposures in mice (50 ppm) and oral gavage doses ranging from 0.1 to 100 mg/kg (Cole et al. 2001; Kenyon et al. 1995; Mathews et al. 1998; Sabourin et al. 1988).

Interroute Extrapolation. The model simulates inhalation and oral exposures and has been applied to predicting internal dose metrics (e.g., amounts of metabolites formed) resulting from exposures by these routes (Cole et al. 2001).

Strengths and Limitations. Strengths of the model are that it simulates disposition of inhaled and ingested benzene, including rates and amounts of major metabolites. Most of the metabolism parameter values were derived empirically from *in vitro* studies, rather than by model optimization. Limitations include: (1) the model has not been evaluated for multiple exposures; (2) the model does not simulate the metabolism of benzene in bone marrow, a major target of benzene toxicity; (3) the model, as configured in Cole et al. (2001), does not simulate benzene disposition in humans.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Benzene is readily absorbed via all natural routes of exposure (inhalation, oral, and dermal) and distributed throughout the body via the blood. Based on physical properties such as slight water solubility, high lipid solubility, and nonpolarity, benzene is expected to enter the blood via passive diffusion from gut, lungs, and skin. Benzene is expected to readily bind to plasma proteins (Travis and Bowers 1989). Being moderately lipophilic, benzene tends to accumulate in fatty tissues. However, benzene metabolism is relatively rapid and required for hematopoietic and leukemogenic effects to be expressed. Multiple reactive metabolites appear to be involved in benzene toxicity. As discussed in detail in Section 3.5.2, potential candidates include benzene oxide, phenolic metabolites (phenol,

catechol, hydroquinone, 1,2,4-benzenetriol, and 1,2- and 1,4-benzoquinone), and *trans,trans*-muconaldehyde. Both human and animal data demonstrate the importance of CYP2E1 in benzene metabolism (see Section 3.4.3 for a detailed discussion). Metabolism is assumed to take place primarily in the liver, with some secondary metabolism in the bone marrow, the site of characteristic benzene toxicity. Processes involved in transport of hepatic metabolites of benzene to the critical toxicity target (bone marrow) are not known, although some degree of covalent binding of reactive benzene metabolites to blood proteins is expected. At relatively low exposure levels, urinary excretion of conjugated benzene derivatives represents the major excretory pathway for benzene. Biliary excretion represents a minor excretory pathway.

3.5.2 Mechanisms of Toxicity

Numerous mechanistic studies have been conducted in an effort to elucidate mechanisms of benzene-induced hematotoxic and leukemogenic effects, widely recognized as the most critical effects of benzene exposure. Conversely, benzene-induced effects on reproduction, development, and the nervous system have not been studied in sufficient detail to assess mechanisms of toxicity for these end points. The database of information for benzene-induced hematotoxic and leukemogenic effects has been reviewed extensively (e.g., Bird et al. 2005; Irons 2000; Morgan and Alvares 2005; Ross 1996, 2000, 2005; Schnatter et al. 2005; Smith 1996a, 1996b; Snyder 2000a, 2000b, 2002; Snyder and Hedli 1996; Snyder and Kalf 1994). It is generally believed that reactive hepatic metabolites of benzene are transported to the major toxicity target (bone marrow). Additional metabolism likely occurs in bone marrow. Phenolic metabolites (phenol, hydroquinone, catechol, 1,2,4-benzenetriol, and 1,2- and 1,4-benzoquinone) appear to play a major role in benzene toxicity. Smith (1996a, 1999b) noted that the phenolic metabolites can be metabolized by bone marrow peroxidases, such as myeloperoxidase (MPO), to highly reactive semiquinone radicals and quinones that stimulate the production of reactive oxygen species. These steps lead to damage to tubulin, histone proteins, topoisomerase II, other DNA associated proteins, and DNA itself (clastogenic effects such as strand breakage, mitotic recombination, chromosome translocations, and aneuploidy). Damage to stem or early progenitor cells would be expressed as hematopoietic and leukemogenic effects.

Results of several mechanistic studies demonstrate that benzene hematotoxicity is dependent upon metabolism (see Section 3.4.3 for a detailed discussion). Inhibition of benzene metabolism reduced its toxicity (Andrews et al. 1977). Partial hepatectomy decreased both benzene metabolism and toxicity (Sammett et al. 1979). Pretreatment with inducers of metabolism increased both benzene metabolism and

toxicity (Gad-El-Karim et al. 1985, 1986). Inhibition of CYPs (enzymes that catalyze oxidation pathways in benzene metabolism) reduced benzene-induced genotoxicity (Tuo et al. 1996). Mice lacking CYP2E1 or microsomal epoxide hydrolase expression were not susceptible to benzene levels known to cause myelotoxicity and cytotoxicity in wild type mice (Bauer et al. 2003; Valentine et al. 1996a, 1996b). Occupationally-exposed workers with a phenotype corresponding to rapid CYP2E1 metabolism were more susceptible to benzene toxicity than those expressing slow CYP2E1 metabolism (Rothman et al. 1997). The enzyme NAD(P)H:quinone oxidoreductase (NQ01), which maintains quinones in reduced form where they are more readily conjugated and excreted (Nebert et al. 2002), is another example of the importance of metabolism in benzene hematotoxicity. Between 22% (Caucasian) and 45% (Asian) of the population is homozygous for an NQ01 allele whereby NQ01 production is negligible. Rothman et al. (1997) found that workers homozygous for an NQ01 allele whereby NQ01 production is negligible (wild type) exhibited a 2.4-fold increased risk for benzene hematotoxicity than workers with the normal genotype. Greater than 7-fold increased risk of benzene hematotoxicity was noted in workers who expressed both rapid CYP2E1 metabolism and the NQ01 wild type (Rothman et al. 1997).

Benzene metabolism involves the production of reactive metabolites that may act directly on cellular macromolecules (proteins and DNA). No single metabolite has been implicated; effects are probably due to many metabolites, which include benzene oxide, reactive products of the phenol pathway (catechol, hydroquinone, and 1,4-benzoquinone), and *trans,trans*-muconaldehyde. Evidence that benzene oxide may play a role in benzene toxicity includes findings that benzene oxide is a product of oxidative benzene metabolism in mouse, rat, and human liver microsomes (Lovern et al. 1997), benzene oxide can be released from the liver into the blood (Lindstrom et al. 1997), benzene oxide-protein adducts have been found in the blood and bone marrow of mice exposed to benzene (McDonald et al. 1994), and benzene oxide hemoglobin and albumin adducts have been detected in the blood of workers exposed to benzene (Rappaport et al. 2002a, 2002b; Yeowell-O'Connell et al. 1998).

Urinary *trans,trans*-muconic acid has been detected in humans and animals following benzene exposure, although its purported reactive precursor (*trans,trans*-muconaldehyde; see Figure 3-3) has not been detected *in vivo*. The highly reactive *trans,trans*-muconaldehyde, which has been found in mouse hepatic microsomes (Latriano et al. 1986), can undergo reductive and oxidative metabolism (Goon et al. 1992) and has been shown to be hematotoxic (Witz et al. 1985). A small amount (<0.05%) of parenterally-administered *trans,trans*-muconaldehyde to mice reached the bone marrow (Zhang et al. 1997). Rivedal and Witz (2005) found *trans,trans*-muconaldehyde to be a strong inhibitor of gap junction intercellular communication in rat liver epithelial cells.

Phenolics (phenol, catechol, hydroquinone; 1,2,4-benzenetriol; 1,2- and 1,4-benzoquinone) are major metabolites of benzene that have been shown to persist in bone marrow following inhalation exposure (Rickert et al. 1979; Sabourin et al. 1988). Hydroquinone induces chromosomal damage in lymphocytes *in vitro* in a manner similar to that observed in lymphocytes of benzene-exposed workers (Eastmond et al. 1994; Stillman et al. 1997; Zhang et al. 1998b). Kolachana et al. (1993) demonstrated that both hydroquinone and 1,2,4-benzenetriol cause oxidative damage to DNA in mouse bone marrow (*in vivo*) and human myeloid cells (*in vitro*). Glutathione adducts of 1,4-benzoquinone have been shown to be hematotoxic in bone marrow of mice exposed to benzene (Bratton et al. 1997). Additional evidence that phenolic metabolites may play an important role in benzene toxicity includes the finding that MPO, an enzyme found in high concentration in bone marrow (Bainton et al. 1971), catalyzes the oxidation of polyphenols to reactive quinones, semiquinones, and oxygen radicals (Nebert et al. 2002). This can lead to strand breaks and inhibition of topoisomerases and microtubule assembly, which could result in chromosome damage (Chen and Eastmond 1995; Eastmond et al. 2001; Irons and Neptune 1980; Smith 1996a, 1996b). Eastmond et al. (2005) demonstrated that hydroquinone can be readily activated to a potent topoisomerase II inhibitor in the presence of human MPO and H_2O_2 and that partial inhibition occurs at hydroquinone concentrations as low as 50 nM. Irons and Neptune (1980) suggested that benzene-derived hydroquinone may inhibit cell replication by covalently binding to tubulin, a protein essential for spindle formation in mitosis.

Benzene-induced effects on DNA have been studied in some detail. Schwartz et al. (1985) demonstrated that benzene metabolites inhibit mitochondrial DNA polymerase. Hydroquinone has been shown to inhibit ribonucleotide reductase, a key step in DNA synthesis (Li et al. 1997, 1998). Rushmore et al. (1984) found that benzene metabolites covalently bind to mitochondrial DNA and inhibit RNA synthesis. Benzene metabolites bound to hepatic DNA have been observed in animals following inhalation exposure to radiolabeled benzene (Lutz and Schlatter 1977). However, the reported levels of DNA adduct formation appear to be low. For example, Creek et al. (1997) observed adducts to both protein and DNA in the range of nanograms per kilogram in mice given radiolabeled benzene.

Benzene-induced DNA damage may result from the oxidation of DNA by reactive oxygen species that are produced during benzene metabolism. Both 1,4-benzoquinone and hydroquinone are known to increase superoxide, nitric oxide, and hydrogen peroxide in HL-60 cells (Rao and Snyder 1995). Chen et al. (2004) observed nitric oxide-derived benzene metabolites, namely nitrobenzene, nitrophenyl, and nitrophenol isomers in the bone marrow of mice 1 hour following intraperitoneal injection of a 400 mg/kg

dose of benzene. These nitro metabolites were either not detected in other tissues or were present in much smaller concentrations, indicating that they were most likely produced in bone marrow. Brunmark and Cadenas (1988) described a metabolic pathway from hydroquinone leading to the production of glutathionyl-benzenetriol, which can undergo autoxidation leading to superoxide formation. Rao (1996) suggested that benzene-induced DNA damage is mediated by the release of free iron in the bone marrow (probably by polyphenolic metabolites), followed by the chelation of iron by hydroquinone or benzenetriol to yield a reactive oxygen-generating species such as superoxide, which causes oxidative damage to DNA.

The expression of benzene toxicity may involve multiple benzene metabolites. All of the known unconjugated metabolites of benzene, with the exception of phenol and 1,2,4-benzenetriol, have been shown to decrease erythropoiesis (Snyder and Hedli 1996). In mice, the combination of phenol and hydroquinone resulted in exacerbated loss of bone marrow cellularity (Eastmond et al. 1987), increased peroxidatic activation of hydroquinone (Subrahmanyam et al. 1989, 1990), and increased DNA damage (Lévay and Bodell 1992; Marrazzini et al. 1994). Combinations of either phenol and hydroquinone, or phenol and catechol, were more hematotoxic than any of the metabolites given alone (Guy et al. 1991). The combination of hydroquinone and muconaldehyde was the most potent in inhibiting erythropoiesis (Snyder et al. 1989). Catechol was found to stimulate the peroxidase-mediated activation of hydroquinone and produced a synergistic genotoxic effect in lymphocytes (Robertson et al. 1991).

3.5.3 Animal-to-Human Extrapolations

Pathways of benzene metabolism are generally similar among various rodent and nonhuman primate species. However, species differences exist regarding capacity to metabolize benzene and relative proportions of various benzene metabolites formed.

Species differences exist in absorption and retention of benzene. For example, following 6-hour exposures to low concentrations (7–10 ppm) of benzene vapors, mice retained 20% of the inhaled benzene, whereas rats and monkeys retained only 3–4% (Sabourin et al. 1987, 1992). Mice exhibit a greater overall capacity to metabolize benzene, compared to rats. An Inhalation exposure to 925 ppm resulted in an internal dose of 152 mg/kg in mice, approximately 15% of which was excreted as parent compound, and an internal dose of 116 mg/kg in rats, approximately 50% of which was excreted unchanged (Henderson et al. 1992; Sabourin et al. 1987).

The proportions of benzene metabolites produced depend on both species and exposure concentration. Hydroquinones and muconic acid (potential sources of benzene toxicity) were detected in much higher concentrations in the blood, liver, lung, and bone marrow of mice than rats, following a 6-hour inhalation exposure to benzene at a concentration of 50 ppm (Sabourin et al. 1988). It is generally understood that metabolic profiles of benzene in mice and humans are more similar than those of humans and rats. Sabourin et al. (1989a) noted increased production of detoxification metabolites (phenylglucuronide and prephenylmercapturic acid) and decreased production of potentially toxic metabolites (hydroquinones and muconic acid) in both mice and rats exposed to benzene at much higher concentrations (600 ppm in air or 200 mg/kg orally), which indicates that extrapolation of toxicological results from studies using high exposure concentrations to low exposure scenarios may result in an underestimation of risk.

Recent PBPK models have tried to address benzene metabolism in an effort to derive animal-to-human extrapolations (Bois et al. 1991a, 1996; Cole et al. 2001; Medinsky 1995; Medinsky et al. 1989a, 1989b, 1989c; Travis et al. 1990). Each model described a multicompartmental model that attempted to relate the generation of metabolites to end points of benzene toxicity. The generation of hydroquinone and muconaldehyde in the liver, with further metabolism in the bone marrow, has been addressed as well as the available data allow. However, the models are not sufficiently refined to allow them to accurately predict human metabolism.

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Thomas and Colborn (1992), was also used in 1996 when Congress mandated the EPA to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), and in 1998, the EDSTAC completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to

the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

No information was located to indicate that benzene may adversely affect the endocrine system.

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6, Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life, and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage

may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water, and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

No clear evidence of age-related differences in susceptibility to benzene toxicity was located. Benzene crosses the placenta and can be found in cord blood at concentrations that equal or exceed those of maternal blood (Dowty et al. 1976). Nursing infants can be exposed to benzene in the breast milk (Fabietti et al. 2004). Limited animal studies indicate that *in utero* exposure to benzene results in hematological changes similar to those observed in animals exposed only as adults (Corti and Snyder 1996; Keller and Snyder 1986, 1988). There is some indication that parental occupational exposure to benzene may play a role in childhood leukemia (Buckley et al. 1989; McKinney et al. 1991; Shaw et al.

1984; Shu et al. 1988). However, none of these studies indicate whether children may be at greater risk than adults for benzene toxicity. Results of Infante-Rivard et al. (2005) indicate that maternal exposure to benzene during pregnancy or from 2 years before pregnancy up to birth does not result in increased risk of childhood acute lymphoblastic leukemia (ALL), a frequent form of childhood cancer (Infante-Rivard et al. 2005). However, no information was located regarding the risk of childhood AML, the form of leukemia most frequently associated with exposure to benzene.

Children could potentially be at increased risk for significant benzene exposure via the inhalation route based on higher activity levels and ventilation rates than adults. However, no information was located to indicate that children are at increased risk for benzene toxicity. Age-related differences in benzene metabolism could potentially affect susceptibility. Results of one human study indicate that CYP2E1, a major enzyme involved in benzene metabolism, is not present in the fetus, but appears in rapidly increasing concentrations during early postnatal development (Vieira et al. 1996). This suggests that fetuses and neonates may be at decreased risk of benzene toxicity due to a reduced metabolic capacity. No information was located regarding potential age-related differences in pharmacodymanic processes such as benzene-target interactions in the hematopoietic system.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the

body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to benzene are discussed in Section 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by benzene are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10, Populations That Are Unusually Susceptible.

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Benzene

Several biomarkers of exposure to benzene have been reported in the literature. Unmetabolized benzene can be detected in the expired air and urine of humans exposed to benzene vapors (Farmer et al. 2005; Fustinoni et al. 2005; Ghittori et al. 1993; Nomiyama and Nomiyama 1974a, 1974b; Sherwood 1988; Srbova et al. 1950; Waidyanatha et al. 2001). Urinary phenol measurements have routinely been used for monitoring occupational exposure to benzene (OSHA 1987), and urinary phenol levels appear to be correlated with exposure levels (Astier 1992; Inoue et al. 1986, 1988b; Karacic et al. 1987; Pagnotto et al. 1961; Pekari et al. 1992).

Urinary *trans,trans*-muconic acid has been widely studied as a biomarker of exposure to benzene (Boogaard and van Sittert 1995, 1996; Ducos et al. 1990, 1992; Inoue et al. 1989b, 2000; Lee et al. 1993; Melikian et al. 1993, 1994; Pezzagno et al. 1999; Popp et al. 1994; Qu et al. 2005; Rothman et al. 1998; Ruppert et al. 1997; Sanguinetti et al. 2001; van Sittert et al. 1993; Weaver et al. 2000). Urinary *S*-phenylmercapturic acid levels have also been correlated with occupational exposure to benzene

(Boogaard and van Sittert 1995, 1996; Farmer et al. 2005; Inoue et al. 2000; Jongeneelen et al. 1987; Popp et al. 1994; Qu et al. 2005). Significant exposure-response trends for urinary *trans,trans*-muconic acid and *S*-phenylmercapturic acid levels have been demonstrated in occupationally-exposed subjects at exposure levels of ≤1 ppm (Qu et al. 2005). The American Conference of Governmental Industrial Hygienists (ACGIH) has established 25 μg *S*-phenylmercapturic acid/g creatinine in the urine and 500 μg *trans,trans*-muconic acid/g creatinine in the urine as Biological Exposure Indices (BEIs) for benzene exposure in the workplace (ACGIH 2006). The BEI is primarily an index of exposure and not a level at which health effects might occur from exposure to benzene. Positive correlations have been made between benzene workplace air levels and urinary catechol and hydroquinone in exposed workers (Inoue et al. 1988a, 1988b; Rothman et al. 1998).

Hemoglobin and albumin adducts of the benzene metabolites, benzene oxide and 1,4-benzoquinone, have been used as biomarkers of exposure to benzene (Bechtold and Henderson 1993; Bechtold et al. 1992a, 1992b; Smith and Rothman 2000; Yeowell-O'Connell et al. 1998, 2001). Furthermore, DNA adducts with benzene metabolites have been found after benzene exposure (Hedli et al. 1991; Lutz and Schlatter 1977; Reddy et al. 1989).

Ong et al. (1995) evaluated various biomarkers of benzene exposure for their relationship with environmental benzene levels. Muconic acid in the urine correlated best with environmental benzene concentrations. Urinary hydroquinone levels were the most accurate biomarker of exposure for the phenolic metabolites of benzene, followed by phenol and catechol. No correlation was found between environmental benzene levels and unmetabolized benzene in the urine, although other studies suggest that benzene in the urine may be a useful biomarker of occupational exposure (Ghittori et al. 1993).

The biomarkers discussed in the preceding paragraphs appear to be adequate indicators of exposure to benzene at relatively high occupational exposure levels, and may serve as biomarkers in acute exposure scenarios involving relatively high levels of benzene. However, some of these biomarkers do not appear to be reliable indicators of environmental exposure to benzene (concentrations below the common industrial standard of 1 ppm TWA). For example, results from data collected on 152 chemical workers showed a linear relationship between the concentration of benzene in breathing zone air (when greater than 10 ppm) and urinary concentrations of catechol and hydroquinone (Inoue et al. 1988a). Workers who had an average work-site exposure of 10 ppm benzene showed no significant differences in the concentration of urinary catechol or hydroquinone when compared to a group of unexposed subjects. In a study of pharmacy workers exposed to benzene levels measured in the parts per billion (ppb) range, there

was no significant difference in urinary levels of *trans,trans*-muconic acid between subjects exposed to 1.5 ppb and those exposed to 2.5 ppb (Sanguinetti et al. 2001). Recent reports indicate that urinary benzene may serve as the most sensitive biomarker of exposure to benzene concentrations well below 1 ppm (Farmer et al. 2005; Fustinoni et al. 2005).

Several additional factors must be taken into account when assessing the reliability of biomarkers of exposure to benzene. High and variable background levels of phenol and its metabolites result from ingestion of vegetables, exposure to other aromatic compounds, ingestion of ethanol, and inhalation of cigarette smoke (Nakajima et al. 1987). Relatively high urinary phenol levels (5–42 mg/L) have been found in persons with no known exposure to benzene (NIOSH 1974). Although muconic acid is used as a marker for benzene exposure, muconic acid in the urine can also result from ingestion of sorbic acid, a common food preservative (Ducos et al. 1990). Inoue et al. (1989b) suggested that individual urinary *trans,trans*-muconic acid content was not a useful index of benzene exposure due to large variations in measured individual background urinary *trans,trans*-muconic acid values.

In summary, several benzene metabolites may serve as biomarkers of exposure to benzene. Urinary benzene appears to be the most sensitive biomarker for low-level exposure to benzene. Refer to Tables 7-1 and 7-2 for information regarding analytical methods for determining benzene in biological samples and benzene metabolites in urine.

3.8.2 Biomarkers Used to Characterize Effects Caused by Benzene

In addition to using levels of benzene and benzene metabolites for monitoring purposes, various biological indices might also be helpful in characterizing the effects of exposure to benzene. As with monitoring for benzene exposure, monitoring for effects may best be accomplished through the use of a series of biomarkers with correlation of the results. Decreases in erythrocyte and leukocyte counts have been used as an indicator of high occupational exposures. Monitoring of benzene workers has included monthly blood counts, with workers being removed from areas of high benzene exposure when leukocyte counts fell below 4,000/mm^3 or erythrocyte counts fell below 4,000,000/mm^3 (ITII 1975; OSHA 1987). Hayes (1992) indicates that benzene-related leukopenia, commonly thought of as an intermediate end point in the process of developing benzene-related leukemia, is considered a biomarker of benzene poisoning in China, not necessarily related to leukemia. Leukocyte alkaline phosphatase (LAP) activity was increased in benzene workers exposed to about 31 ppm for a chronic time period (Songnian et al. 1982). Increased LAP activity is an indicator of myelofibrosis and is associated with both decreased

white blood cell counts and changes in bone marrow activity. The change in LAP activity could be used in the diagnosis of benzene poisoning since it was more sensitive than the change in the leukocyte count, although it is not a biomarker that is specific for benzene. Additionally, it seems reasonable that chromosomal aberrations in bone marrow and peripheral blood lymphocytes and sister chromatid exchanges could be used to monitor for benzene effects (Eastmond et al. 1994; Van Sittert and de Jong 1985). Benzene metabolites have also been found to form adducts with DNA (Chenna et al. 1995; Lutz and Schlatter 1977; Norpoth et al. 1988; Rushmore et al. 1984; Snyder et al. 1987).

Exposure to benzene causes toxic effects in the bone marrow via its metabolites and possibly by benzene via solvent effects (Eastmond et al. 1987; Gad-El-Karim et al. 1985; Hedli et al. 1991; Irons et al. 1980). Therefore, it is possible that hematological tests could be used as markers of hematotoxicity. To date, surveillance and early diagnosis of benzene hematotoxicity rely primarily on the complete blood count, including hemoglobin, hematocrit, erythrocyte count, leukocyte count, and differential and platelet counts. In effect, complete blood counts and marrow exams should be good for early detection of preleukemic lesions. Additionally, cytogenetic tests of marrow cells are being used. Workers exposed to benzene in the air have shown elevated levels of delta-aminolevulinic acid in erythrocytes and elevated coproporphyrin in the urine (Kahn and Muzyka 1973). These may be biomarkers for disruption of porphyrin synthesis and may be early indicators of adverse hematological effects. These effects are not specific for benzene. Hedli et al. (1991) observed that in rats, benzene metabolite-DNA adducts were observed in the bone marrow at doses that did not affect bone marrow cellularity, and suggested that monitoring of the bone marrow DNA adducts might be a sensitive bioassay of genotoxic effects of benzene exposure. Work by other researchers also suggests that monitoring DNA adducts or products of DNA damage might be useful (Bodell et al. 1993; Chen et al. 1994; Lagorio et al. 1994a; Reddy et al. 1989).

3.9 INTERACTIONS WITH OTHER CHEMICALS

Studies have been conducted on the interaction of benzene with other chemicals, both *in vivo* and in the environment. Benzene metabolism is complex, and various xenobiotics can induce or inhibit specific routes of detoxification and/or activation in addition to altering the rate of benzene metabolism and clearance from the body. Toluene, Aroclor 1254, phenobarbital, acetone, and ethanol are known to alter the metabolism and toxicity of benzene. Interactions reported in *in vivo* studies occurred at relatively high benzene exposure levels, which would not likely be encountered near hazardous waste sites.

Ethanol and benzene induce the formation of the hepatic cytochrome P-450 isoenzyme, CYP2E1, in rabbits and rats (Gut et al. 1993; Johansson and Ingelman-Sundberg 1988), although benzene derivatives, such as toluene and xylene, can inhibit the enzymatic activity of the isozyme (Koop and Laethem 1992). Ethanol enhances both the metabolism (*in vitro*) and the toxicity (*in vivo*) of benzene in animals (Baarson et al. 1982; Nakajima et al. 1985). Administration of ethanol (5 or 15% in drinking water, 4 days/week for 13 weeks) to mice exposed to benzene vapors at a concentration of 300 ppm, 6 hours/day, 5 days/week for 13 weeks) resulted in greater severity of benzene-induced hematological effects (anemia, lymphocytopenia, bone marrow aplasia, transient increases in normoblasts and peripheral blood atypia) relative to benzene-exposed mice not given ethanol (Baarson et al. 1982). The modulating effects of benzene were dose dependent. The enhancement of the hematotoxic effects of benzene by ethanol may be of particular concern for benzene-exposed workers who consume alcohol (Nakajima et al. 1985), although the interactions demonstrated in the mice occurred at much higher benzene exposure concentrations than would likely be experienced in workplace air. Benzene can interfere with the disappearance of ethanol from the body. Accordingly, increased central nervous system disturbances (e.g., depression) may occur following concurrent exposure to high levels of benzene and ethanol.

Other chemicals that induce specific isoenzymes of cytochrome P-450 can increase the rate of benzene metabolism and may alter metabolism pathways favoring one over another. Ikeda and Ohtsuji (1971) presented evidence that benzene hydroxylation was stimulated when rats were pretreated with phenobarbital and then exposed to 1,000 ppm of benzene vapor for 8 hours/day for 2 weeks. Additionally, phenobarbital pretreatment increased the rate of metabolism by 40% in rats and 70% in mice (Pawar and Mungikar 1975). In contrast, rats exposed to phenobarbital showed no effects on the metabolism of micromolar amounts (35–112.8 μmol) of benzene *in vitro* (Nakajima et al. 1985).

Co-administration of toluene inhibited the biotransformation of benzene to phenol in rats (Ikeda et al. 1972; Inoue et al. 1988b). This was due to competitive inhibition of the oxidation mechanisms involved in the metabolism of benzene. Phenobarbital pretreatment of the rats alleviated the suppressive effect of toluene on benzene hydroxylation by the induction of oxidative activities in the liver. This effect has been observed in other studies in rats (Purcell et al. 1990).

Mathematical models of benzene and phenol metabolism suggest that the inhibition by benzene of phenol metabolism, and by phenol on benzene metabolism, occurs through competition for a common reaction site, which can also bind catechol and hydroquinone (Schlosser et al. 1993). Flavonoids have been shown

to inhibit phenol hydroxylase or increase phenol hydroxylase activity in a dose-dependent manner, dependent on the oxidation potential of the flavonoid (Hendrickson et al. 1994).

SKF-525A and carbon monoxide are classic inhibitors of cytochrome P-450s. The binding between P-450 and carbon monoxide or SKF-525A is coordinate covalent. Carbon monoxide inhibits all cytochrome P-450 isoenzymes since it binds to the heme component of cytochrome P-450, whereas SKF-525A inhibits specific types. SKF-525A inhibited benzene metabolism in the rat (Ikeda et al. 1972). Injection of 80 mg/kg of SKF-525A in rats resulted in a depression of phenol excretion. It also prolonged phenol excretion and interfered in the conversion of benzene to glucuronides and free phenols. Carbon monoxide, aniline, aminopyrine, cytochrome C, and metyrapone inhibited benzene metabolism *in vitro* by mouse liver microsomes (Gonasun et al. 1973).

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to benzene than will most persons exposed to the same level of benzene in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of benzene, or compromised function of organs affected by benzene. Populations who are at greater risk due to their unusually high exposure to benzene are discussed in Section 6.7, Populations with Potentially High Exposures.

Variability in human susceptibility to benzene toxicity may be related, at least in part, to genetic polymorphisms associated with metabolic processes. As discussed in Section 3.4.3, the flavoenzyme, NAD(P)H:quinone oxidoreductase (NQ01), catalyzes the reduction of 1,2- and 1,4-benzoquinone (reactive metabolites of benzene) to catechol and hydroquinone, respectively (Nebert et al. 2002), thus protecting cells from oxidative damage by preventing redox cycling. The NQ01*1 (wild type) allele codes for normal NQ01 enzyme and activity. An NQ01*2 allele encodes a nonsynonymous mutation that has negligible NQ01 activity. Approximately 5% of Caucasians and African Americans, 15% of Mexican-Americans, and 20% of Asians are homozygous for the NQ01*2 allele (Kelsey et al. 1997; Smith and Zhang 1998). Rothman et al. (1997) demonstrated that workers expressing negligible NQ01 activity were at increased risk of benzene poisoning (Rothman et al. 1997). In the same group of workers, those expressing rapid CYP2E1 activity were also at increased risk of benzene poisoning. Those workers with polymorphisms for both negligible NQ01 activity and rapid CYP2E1 activity exhibited greater than 7-fold increased risk of benzene poisoning than workers not expressing these polymorphisms. These

results indicate that individuals expressing rapid CYP2E1 activity may also be at increased risk for benzene toxicity.

Polymorphisms have been described for many of the glutathione genes (Cotton et al. 2000; Hengstler et al. 1998; Strange and Fryer 1999), the myeloperoxidase gene (Williams 2001), and the epoxide hydrolase gene (Omiecinski et al. 2000), which are known to be involved in benzene metabolism. However, the potential involvement of such polymorphisms in benzene toxicity have not been demonstrated in benzene-exposed workers.

Individuals with medical conditions that include reduced bone marrow function or decreased blood factors would be at increased risk for benzene toxicity. Treatments for certain medical conditions might result in decreases in particular blood factors, which could lead to increased susceptibility to benzene poisoning.

Ethanol can increase the severity of benzene-induced anemia, lymphocytopenia, and reduction in bone marrow cellularity, and produce transient increases in normoblasts in the peripheral blood and atypical cellular morphology (Baarson et al. 1982). The enhancement of the hematotoxic effects of benzene by ethanol is of particular concern for benzene-exposed workers who consume alcohol (Nakajima et al. 1985). Accordingly, increased central nervous system disturbances (e.g., depression) may be expected following concurrent exposure to benzene and ethanol.

Gender-related differences in susceptibility to benzene toxicity have been observed in animals. For example, Kenyon et al. (1998) exposed male and female mice to benzene vapor concentrations of 100 or 600 ppm and found increased benzene metabolism and associated genotoxicity in males, relative to females. Brown et al. (1998) used a PBPK modeling approach to assess potential gender-related differences in susceptibility to benzene. Their results suggest that women exhibit a higher blood/air partition coefficient and maximum velocity of benzene metabolism than men, and that women metabolize 23-26% more benzene than men under similar exposure scenarios. However, gender-related differences in susceptibility among benzene-exposed workers were not located in available reports.

At early stages of human development, metabolic pathways may not be fully functional, which might result in a lower level of susceptibility. Young children might experience increased susceptibility to benzene by inhalation due to increased breathing rates and potential for increased absorption, relative to

adults. However, no definitive human or animal data were located regarding age-related differences in susceptibility to benzene.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to benzene. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to benzene. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide specific information about treatment following exposures to benzene:

Goldfrank LR, Flomenbaum NE, Lewin NA, et al., eds. 1998. Goldfrank's toxicologic emergencies. 6th ed. Stamford: Appleton & Lange, 1384-1398.

Haddad LM, Shannon MW, Winchester JF. 1998. Clinical management of poisoning and drug overdose. 3rd ed. Philadelphia, PA: W.B Saunders Company, 940-941.

3.11.1 Reducing Peak Absorption Following Exposure

Human exposure to benzene can occur by inhalation, oral, or dermal routes. General recommendations for reducing absorption of benzene following acute high-level inhalation exposure have included moving the patient to fresh air and monitoring for respiratory distress (Bronstein and Currance 1988; Haddad et al. 1998; HSDB 2007; Kunisaki and Augenstein 1994; Stutz and Janusz 1988). The administration of 100% humidified supplemental oxygen with assisted ventilation, when required, has also been suggested (Bronstein and Currance 1988; Haddad et al. 1998; Kunisaki and Augenstein 1994; Stutz and Janusz 1988). In the case of eye exposure, irrigation with copious amounts of water or saline has been recommended (Bronstein and Currance 1988; Haddad et al. 1998; HSDB 2007; Stutz and Janusz 1988). The removal of contaminated clothing and a thorough washing of exposed areas with soap and water have also been recommended (Bronstein and Currance 1988; Ellenhorn and Barceloux 1988; Haddad et al. 1998; Kunisaki and Augenstein 1994; Stutz and Janusz 1988). Some sources suggest the administration of water or milk to the victim after ingestion of benzene (Stutz and Janusz 1988). Emesis may be indicated following recent substantial ingestion of benzene (Ellenhorn and Barceloux 1988); however, other sources do not recommend the use of emetics because of the risk of aspiration pneumonitis, especially once the patient loses consciousness (Bronstein and Currance 1988; Haddad et al. 1998). Some sources recommend gastric lavage if indicated (Haddad et al. 1998; Kunisaki and Augenstein 1994).

While lavage may be useful, induction of emesis should be regarded with great caution because the rapid onset of central nervous system depression may lead to aspiration. Although of unproven value, administration of a charcoal slurry, aqueous or mixed with saline cathartic or sorbitol, has also been suggested as a way to stimulate fecal excretion of the chemical before it is completely absorbed by the body (HSDB 2007; Kunisaki and Augenstein 1994; Stutz and Janusz 1988). Diazepam and phenytoin may be helpful in controlling seizures (Bronstein and Currance 1988; HSDB 2007; Stutz and Janusz 1988). Administration of epinephrine or other catecholamines has not been recommended because of the possibility of myocardial sensitization and subsequent arrhythmia (Haddad et al. 1998; HSDB 2007; Nahum and Hoff 1934).

3.11.2 Reducing Body Burden

Following absorption into the blood, benzene is rapidly distributed throughout the body. Since benzene is lipophilic, it is preferentially distributed to lipid-rich tissues. The initial stage of benzene metabolism is the formation of benzene oxide via P-450 mixed-function oxidases. Detoxification pathways generally involve the formation of glutathione conjugates of benzene oxide and glucuronide or sulfate conjugates of phenol or its subsequent metabolites, catechol, hydroquinone, and trihydroxybenzene. Other metabolites of benzene also have known toxic effects. Exhalation is the main route for excretion of unmetabolized benzene, while metabolized benzene is excreted primarily in the urine. Studies in humans and animals indicate that both exhalation and urinary excretion occur in several phases, with half-lives of minutes to hours. Hence, benzene and its metabolites have relatively short half-lives in the body, and while some of these metabolites are clearly toxic, accumulation of substantial body burdens are not expected.

No methods are currently used for reducing the body burden of benzene. It is possible that methods could be developed to enhance the detoxification and elimination pathways, such as ensuring sufficient glutathione stores in the body by the administration of N-acetyl-L-cysteine.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

Administration of indomethacin, a nonsteroidal anti-inflammatory drug, has been shown to prevent benzene-induced myelotoxicity in mice and the accompanying increase in prostaglandin E in the bone marrow (Kalf et al. 1989; Renz and Kalf 1991). Co-administration of indomethacin also prevented an increase in the number of micronucleated polychromatic erythrocytes in peripheral blood. The authors suggest that these results suggest a role for prostaglandin synthetase in benzene-induced myelotoxic and genotoxicity, and a way to interfere with that process with substances such as indomethacin.

Prostaglandins have been shown to inhibit hematopoiesis (Kalf et al. 1989). Additionally, prostaglandin synthetase could be involved in the oxidation of phenol and/or hydroquinone to toxic metabolites (Kalf et al. 1989).

The use of indomethacin to block benzene toxicity has led to data that indicate that myelotoxicity may involve the destruction of stromal macrophages that produce IL-1, a cytokine essential for hematopoiesis (Renz and Kalf 1991). External administration of recombinant IL-1 to mice prior to benzene administration prevents the myelotoxicity, presumably by providing a source of the cytokine (Renz and Kalf 1991). Further indication that IL-1 is affected by benzene exposure comes from the work of Carbonnelle et al. (1995), who showed that exposure of human monocytes to micromolar amounts of hydroquinone for 2 hours resulted in significantly decreased secretion of IL-1α and IL-1β at concentrations of 5 μM and above. RNA and protein synthesis were also inhibited. Additional research in this area indicates that tumor necrosis factor may provide protection against the inhibitory effects of hydroquinone on human hematopoietic progenitor cells (Colinas et al. 1995). The research of Shankar et al. (1993) determined that pretreatment with Protein A, a glycoprotein that acts as a multipotent immunostimulant, modulated the toxicity of benzene. Groups of six female albino rats (Swiss Wistar) were injected intraperitoneally with 1.0 mL/kg/body weight (879 mg/kg) benzene once daily for 3 consecutive days. Another group (six per group) was administered intravenously with 60 μg/kg Protein A twice weekly for 2 weeks and then injected with 879 mg/kg benzene intraperitoneally once daily for 3 consecutive days. Controls were injected with normal saline. All of the animals were killed 24 hours after receiving the last benzene injection. Blood was collected from the jugular vein for enumeration of total leukocyte counts. Routine autopsy was performed on all animals and the liver, thymus, spleen, and kidney organs were collected for organ weights. In benzene-only treated animals, there was a significant decrease in the total leukocyte counts in the peripheral blood as well as a significant decrease in the number of lymphocytes, a decrease in the gross organ weights of thymus and spleen, a significant increase in the iron content and lipid peroxidation of the liver and bone marrow, and an increase in low molecular weight iron in the bone marrow. Pretreatment with Protein A prevented these parameters from changing.

3.12 ADEQUACY OF THE DATABASE

Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of benzene is available. Where adequate information is not

available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of benzene.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

3.12.1 Existing Information on Health Effects of Benzene

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to benzene are summarized in Figure 3-6. The purpose of this figure is to illustrate the existing information concerning the health effects of benzene. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need". A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Virtually all of the information regarding health effects in humans comes from studies of workers exposed to benzene-containing solvents and/or adhesives. Exposures to benzene occurred at rotogravure printing shops; at shoe, rubber, and raincoat manufacturing plants; and during chemical manufacturing processes. Case reports and cohort studies describe both acute and chronic health effects. The predominant route of exposure in these studies is inhalation. Dermal contact is also suspected as a possible route of exposure in these studies.

As seen in Figure 3-6, inhalation information for humans is available regarding death; acute-, intermediate-, and chronic-duration systemic effects; immunologic, neurologic, reproductive, developmental, and genotoxic effects; and cancer. However, as mentioned above, human exposure to

Figure 3-6. Existing Information on Health Effects of Benzene

	Death	Acute	Intermediate	Chronic	Immunologic/Lymphoretic	Neurologic	Reproductive	Developmental	Genotoxic	Cancer
	Systemic									
Inhalation	●	●	●	●	●	●	●	●	●	●
Oral	●	●	●			●				●
Dermal	●	●								

Human

	Death	Acute	Intermediate	Chronic	Immunologic/Lymphoretic	Neurologic	Reproductive	Developmental	Genotoxic	Cancer
	Systemic									
Inhalation	●	●	●	●	●	●	●	●	●	●
Oral	●	●	●	●	●	●	●	●	●	●
Dermal		●	●							●

Animal

● Existing Studies

benzene in specific work environments probably occurs not only by inhalation, but also by the dermal route. Limited information is available regarding direct skin contact with benzene by humans. Additionally, oral studies in humans are limited to isolated case reports of death and acute-duration systemic effects subsequent to accidental or intentional ingestion of benzene, although one study (Hunting et al. 1995) described effects in vehicle maintenance workers who siphoned gasoline by mouth. There is limited information on effects of dermal exposure of humans to benzene, including death, acute-duration effects, and cancer.

Inhalation and oral studies in animals provide data on death; systemic effects after acute-, intermediate-, and chronic-duration exposure; and immunologic, neurologic, reproductive, developmental, genotoxic, and cancer effects. Furthermore, data exist regarding acute- and intermediate-duration systemic effects and cancer in animals after dermal exposure to benzene.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. There are reports on the health effects resulting from acute exposure of humans and animals to benzene via the inhalation, oral, and dermal routes. The primary target organs for acute exposure are the hematopoietic system, nervous system, and immune system. Acute effects on the nervous system and immune system are discussed below under Neurotoxicity and Immunotoxicity. Information is also available for levels that cause death in humans (e.g., Cronin 1924; Flury 1928; Greenburg 1926; Tauber 1970; Thienes and Haley 1972) and in animals (e.g., Cornish and Ryan 1965; Drew and Fouts 1974; Smyth et al. 1962) following inhalation and oral exposures.

No acute human or animal data on hematological effects from oral or dermal exposure are available. However, there are acute inhalation data that characterize the effects of benzene on the hematological system in humans and animals. Data regarding effects on the human hematological system following acute inhalation exposure to benzene are scant, but indicate leukopenia, anemia, and thrombocytopenia after more than 2 days of occupational exposure to more than 60 ppm benzene (Midzenski et al. 1992). Data for hematological effects in animals after acute-duration inhalation exposure are more extensive. Changes in peripheral erythrocytes (Chertkov et al. 1992; Cronkite et al. 1985; Rozen et al. 1984; Ward et al. 1985), in peripheral leukocytes (Aoyama et al. 1986; Chertkov et al. 1992; Gill et al. 1980; Green et al. 1981b; Li et al. 1986; Ward et al. 1985; Wells and Nerland 1991), and in bone marrow cells (Chertkov et al. 1992; Corti and Snyder 1996; Cronkite et al. 1989; Dempster and Snyder 1991; Gill et al. 1980; Green et al. 1981b; Neun et al. 1992; Toft et al. 1982) were seen in rats and mice. An acute-duration inhalation

MRL of 0.009 ppm was determined based on the LOAEL for immunologic effects in the mouse (Rozen et al. 1984) (discussed below under Immunotoxicity). No acute-duration oral studies were suitable for deriving MRLs. Additional studies that include dose-response information on hematological effects following acute oral exposure could be designed to provide information that could be useful in deriving an acute-duration oral MRL for benzene. Such studies could be designed to serve as validation for existing PBPK models. Acute dermal exposure at levels that are likely to be found in the environment and at hazardous waste sites is not likely to cause adverse health effects.

Intermediate-Duration Exposure. There is sufficient information in humans and animals to identify the hematopoietic, nervous, and immunological systems as targets for benzene toxicity. The effects on the nervous system and immune system are discussed in the sections below titled Neurotoxicity and Immunotoxicity. Data on adverse hematological effects in humans are available following intermediate-duration exposures to benzene in the workplace (Aksoy and Erdem 1978; Aksoy et al. 1972). However, the exposure levels and durations were not well defined and, therefore, could not be used to calculate an MRL. Studies in rats and mice following inhalation exposure can be used to define NOAELs and LOAELs for hematological and immunological effects (e.g., Baarson et al. 1984; Cronkite et al. 1982, 1985, 1989; Dow 1992; Farris et al. 1993, 1997a, 1997b; Green et al. 1981a, 1981b; Luke et al. 1988b; Plappert et al. 1994a, 1994b; Rosenthal and Snyder 1987; Seidel et al. 1989; Snyder et al. 1978a, 1980; Toft et al. 1982; Vacha et al. 1990; Ward et al. 1985; Wolf et al. 1956). An intermediate-duration inhalation MRL of 0.006 ppm was derived, based on a LOAEL for immunologic effects in the mouse (Rosenthal and Snyder 1987) (discussed below under Immunotoxicity). Data on hematological effects from oral exposures in animals are also available (Hsieh et al. 1988b, 1990, 1991; NTP 1986; Shell 1992; Wolf et al. 1956), but no intermediate-duration oral MRL could be derived because the threshold for hematological (and immunological) effects could not be identified. Additional studies that include dose-response information on hematological effects following intermediate-duration oral exposure could be designed to provide information that could be useful in deriving an intermediate-duration oral MRL for benzene. Such studies could be designed to serve as validation for existing PBPK models. Intermediate-duration dermal exposure at levels that are likely to be found in the environment and at hazardous waste sites is not likely to cause adverse health effects.

Chronic-Duration Exposure and Cancer. The primary target for adverse systemic effects of benzene following chronic exposure is the hematological system. Hematotoxicity was reported in studies of humans chronically exposed to benzene in the workplace air (Aksoy and Erdem 1978; Aksoy et al. 1971, 1972, 1974, 1987; Cody et al. 1993; Dosemeci et al. 1996; Doskin 1971; Erf and Rhoads 1939;

Goldwater 1941; Greenburg et al. 1939; Kipen et al. 1989; Lan et al. 2004a, 2004b; Li et al. 1994; Qu et al. 2002, 2003a, 2003b; Rothman et al. 1996a, 1996b; Townsend et al. 1978; Ward et al. 1996; Yin et al. 1987c). Several studies of occupational inhalation exposure to benzene did not find clinically-defined hematological effects (Collins et al. 1991, 1997; Tsai et al. 1983, 2004), but they had a high degree of uncertainty regarding estimations of benzene exposure levels. The study of workers of shoe manufacturing industries in Tianjin, China (Lan et al. 2004a, 2004b) identified the lowest LOAEL for hematotoxicity and was selected as the principal study for deriving a chronic-duration inhalation MRL of 0.003 ppm for benzene. Chronic-duration animal studies are available for hematological effects following inhalation exposure and provide support to the human data (Snyder et al. 1978a, 1980, 1982, 1984). No human data are available to evaluate hematological effects following oral exposure. Although chronic duration oral animal studies are available for hematological effects (Huff et al. 1989; Maltoni et al. 1983, 1985; NTP 1986), the most extensive study (NTP 1986) did not conclusively define a NOAEL or less serious LOAEL for end points that could be used to derive an MRL. However, a chronic-duration oral MRL of 0.0005 mg/kg/day was derived for benzene based on route-to-route extrapolation of the same results (Lan et al. 2004a, 2004b) that served as the basis for the chronic-duration inhalation MRL. Additional chronic-duration oral animal data could be designed to provide support to the chronic-duration oral MRL and to assist in defining threshold levels for populations living near hazardous waste sites. Dermal data for humans and animals were not available. However, chronic-duration dermal exposure at levels that are likely to be found in the environment and at hazardous waste sites is not likely to cause adverse health effects.

EPA, IARC, and the Department of Health and Human Services have concluded that benzene is a human carcinogen based on sufficient data in humans supported by animal evidence (IARC 2004; IRIS 2007; NTP 2005). Epidemiological studies and case reports provide clear evidence of a causal relationship between occupational exposure to benzene and the occurrence of acute myelogenous leukemia (AML) (Hayes et al. 1997; IARC 1982, 1987; EPA 1995, 1998; IRIS 2007; Rinsky et al. 1987, 2002; Yin et al. 1996a, 1996b), as well as is suggestive evidence of associations between benzene and non-Hodgkin's lymphoma (NHL) and multiple myeloma (Hayes et al. 1997; Rinsky et al. 1987). Studies of workers in Ohio (the Pliofilm study) (e.g., Rinsky et al. 1981, 1987; 2002) and China (the NCI/CAPM study) (e.g., Hayes et al. 1997; Yin et al. 1996a, 1996b) provide the strongest data on the leukemogenic potential of benzene, including exposure-response information, and data from the Pliofilm study was used as the basis of inhalation and oral cancer risk values for benzene (EPA 1998; IRIS 2007). Additional studies on these and other cohorts could better characterize exposure level and exposure duration relationships for benzene

and leukemia, particularly at low levels of exposure, and clarify the potential of benzene to induce NHL and multiple myeloma.

Benzene is a multiple site carcinogen in rats and mice following inhalation exposure (Cronkite 1986; Cronkite et al. 1984, 1985, 1989; Farris et al. 1993; Maltoni et al. 1982a, 1982b, 1983, 1985, 1989; Snyder et al. 1980) and oral exposure (Huff et al. 1989; Maltoni et al. 1983, 1985, 1989; NTP 1986), inducing lymphomas and other neoplasms in numerous tissues not affected in humans. Although contributing to the weight of evidence for carcinogenicity, the animal studies do not identify a suitable model for leukemia in humans. An appropriate animal model would help to provide a better understanding of how benzene causes cancer, particularly the mechanism of benzene leukemogenesis. The exact mechanism of benzene carcinogenicity is not known, but it has been postulated that some benzene metabolites are capable of forming adducts with DNA and are responsible for reduced immune function which could potentially lead to cancer. The clastogenic properties of benzene may play a role in its carcinogenicity. DNA adduct formation could occur with both inhalation and oral exposures (Ding et al. 1983; Sasiadek et al. 1989). Questions that need to be answered with regard to the mechanism of benzene carcinogenesis include how benzene metabolites produce greater-than-additive effects, determination of the critical target genes, whether aplastic anemia is essential to the development of leukemia, and determination of the role of cytokines and growth factor pathways in benzene toxicity.

Genotoxicity. Evidence for the genotoxicity of benzene in humans comes from studies of chronically-exposed populations (Andreoli et al. 1997; Bogadi-Šare et al. 1997; Ding et al. 1983; Forni and Moreo 1967, 1969; Forni et al. 1971a; Hallberg et al. 1996; Hartwich et al. 1969; Hedli et al. 1991; Karacic et al. 1995; Kašuba et al. 2000; Liu et al. 1996; Major et al. 1992, 1994; Nilsson et al. 1996; Picciano 1979; Pitarque et al. 1996, 1997; Popp et al. 1992; Qu et al. 2003a, 2003b; Rothman et al. 1995; Sardas et al. 1994; Sasiadek et al. 1989; Sellyei and Kelemen 1971; Smith et al. 1998; Sul et al. 2002; Tompa et al. 1994; Tough and Court Brown 1965; Tough et al. 1970; Türkel and Egeli 1994; Van den Berghe et al. 1979; Yardley-Jones et al. 1990; Zhang et al. 1998b, 1999). These exposures have occurred primarily via inhalation, although some dermal exposure cannot be ruled out. In spite of the lack of accurate exposure data, exposure to multiple chemicals, and often inappropriate control groups, the association between benzene exposure and the appearance of structural and numerical chromosome aberrations in human lymphocytes suggests that benzene can be considered a human clastogen. Benzene-induced cytogenetic effects, including chromosome and chromatid aberrations, sister chromatid exchanges, and micronuclei, have been consistently found in *in vivo* animal studies (Anderson and Richardson 1981; Au et al. 1991; Chen et al. 1994; Chang et al. 2005; Eastmond et al. 2001; Erexson et al. 1986; Farris et al. 1996; Fujie et

al. 1992; Healy et al. 2001; Lee et al. 2005; Kolachana et al. 1993; Ranaldi et al. 1998; Siou et al. 1981; Toft et al. 1982; Ward et al. 1992). Binding of benzene and/or its metabolites to DNA, RNA, and proteins has been consistently observed in rats and mice (Arfellini et al. 1985; Creek et al. 1997; Lévay et al. 1996; Mani et al. 1999; Mazullo et al. 1989; Turteltaub and Mani 2003). Inhalation exposure of mice has yielded identifiable benzene-derived hemoglobin adducts (Sabourin et al. 1990). Ward et al. (1992) have shown that intermediate-duration exposure of mice to benzene by inhalation at levels below the current PEL may induce gene mutations in the lymphocytes.

Standard short-term *in vitro* assays indicate that benzene's genotoxicity is derived primarily from its metabolites, although benzene has been shown to produce DNA breaks in Chinese hamster ovary cells independent of metabolic activators (Douglas et al. 1985; Eastmond et al. 1994; Lakhanisky and Hendrickx 1985; Zhang et al.1993). There is good evidence that benzene affects cell cycle progression, RNA and DNA synthesis, as well as DNA binding (Forni and Moreo 1967, 1969; Hartwich et al. 1969; Sellyei and Kelemen 1971; Van den Berghe et al. 1979). Chen and Eastmond (1995) showed that benzene metabolites can adversely affect human topoisomerases (enzymes involved in DNA replication and repair); however, evidence exists that some repair of DNA binding with benzene metabolites occurs in human cells (Chenna et al. 1995).

The genotoxicity of benzene has been extensively studied and demonstrated in both humans and laboratory animals. Although some information is available regarding possible mechanisms of benzene genotoxicity and carcinogenicity, additional mechanistic studies would be helpful to more completely characterize mechanisms responsible for these effects.

Reproductive Toxicity. Reproductive data are available on women occupationally exposed to benzene (Mukhametova and Vozovaya 1972; Vara and Kinnunen 1946). The data suggest spontaneous abortions, menstrual disturbances, and ovarian atrophy. These studies are limited by the difficulty in identifying appropriate controls, problems in controlling for concomitant exposures to other chemicals, and inadequate follow-up. Only one study was found that described the reproductive effects on men exposed to benzene (Stucker et al. 1994). There are some data available from inhalation studies on reproductive effects of benzene in animals. Although there are data on adverse gonadal effects (e.g., atrophy/degeneration, decrease in spermatozoa, moderate increases in abnormal sperm forms) (Ward et al. 1985; Wolf et al. 1956), data on reproductive outcomes are either negative (Coate et al. 1984; Green et al. 1978; Kuna et al. 1992; Murray et al. 1979; Tatrai et al. 1980a) or inconclusive or conflicting (i.e., number of live fetuses, incidences of pregnancies) (Murray et al. 1979; Ungvary and Tatrai 1985).

Gofmekler (1968) showed infertility in female rats after intermediate-duration inhalation exposure to 210 ppm, but the results are poorly reported. Thus, they provide only suggestive evidence that benzene may have an adverse effect on reproductive outcomes. No data were located on reproductive effects following oral exposure to benzene in humans. Negative effects on reproductive outcome have been reported in one oral study in animals (Exxon 1986). NTP (1986) reported neoplastic changes in the reproductive tissues of female rats and male and female mice after chronic oral exposure. Given the paucity of available data across all exposure routes, it would be useful to have additional 90-day studies conducted by the oral and inhalation routes that assess reproductive organs histologically or cytogenetically. Although dermal contact is not likely to be the most relevant route of exposure for humans at hazardous waste sites, data on dermal exposure would also be useful. If the results of the suggested inhalation or oral studies indicate reproductive toxicity, multigeneration or continuous breeding studies for oral and inhalation exposures would help clarify the potential for benzene to cause reproductive effects in humans.

Developmental Toxicity. Based on epidemiological studies at hazardous waste sites, an increased susceptibility to benzene of pregnant women or their offspring has not been demonstrated (Budnick et al. 1984; Goldman et al. 1985; Heath 1983; Olsen 1983). However, these studies have several limitations that make it impossible to assess the effect of benzene on the human fetus. For example, the few studies that do exist are limited by a lack of control incidences for end points, problems in identifying exposed populations, a lack of data on exposure levels, and/or exposure to multiple substances (Budnick et al. 1984; Forni et al. 1971a; Funes-Cravioto et al. 1977; Goldman et al. 1985; Heath 1983; Olsen 1983). In the occupational setting, however, there may be stronger evidence of increased susceptibility to benzene of pregnant women and/or their offspring (Forni et al. 1971a; Funes-Cravioto et al. 1977). For example, severe pancytopenia and increased chromosomal aberrations occurred in a pregnant worker exposed to benzene (levels not reported) during the entire pregnancy. She gave birth to a healthy boy (Forni et al. 1971b). On the other hand, increased frequency of chromatid and isochromatid breaks and sister chromatid exchanges was found in lymphocytes from 14 children of female workers exposed to benzene (levels not reported) and other organic solvents during pregnancy (Funes-Cravioto et al. 1977). Irrespective of the hematological effects reported in the pregnant worker or the 14 children of female workers, the lack of complete and detailed medical records and the lack of follow-up limit the significance of effects with regard to post exposure morbidity.

A number of investigations have evaluated the developmental/maternal toxicity of benzene in animals following inhalation exposures. In these investigations, fetotoxicity was evidenced by reduced fetal

weight and/or minor skeletal variations at concentrations 47 ppm (Coate et al. 1984; Green et al. 1978; Kimmel and Wilson 1973; Kuna and Kapp 1981; Murray et al. 1979; Tatrai et al. 1980a, 1980b; Ungvary and Tatrai 1985). However, persistently altered fetal hematopoiesis occurred in mice at 20 ppm (Keller and Snyder 1986, 1988). Additional information designed to assess the hematopoietic system of the developing fetus (human or animal) following low-level *in utero* exposures to benzene is needed. Oral data are limited to two animal studies in which benzene was shown to reduce pup body weight when mice were administered a high single oral dose (Seidenberg et al. 1986) or have no effect after gestational exposure (Exxon 1986). Additional oral studies designed to assess the developmental effects (human or animal) of low-level exposures to benzene would be useful. No data are available on the developmental toxicity of benzene following dermal exposure. Although these data would be useful, the most likely routes of exposure for humans at hazardous waste sites are the inhalation and oral routes.

Immunotoxicity. The immune system is known to be a target for benzene toxicity. The effects do not appear to be route- or species-specific. The evidence for immunotoxicity in humans comes from workers exposed by inhalation of intermediate and chronic durations. A series of studies demonstrated decreases in numbers of circulating leukocytes (Aksoy et al. 1971, 1972, 1974), but levels of exposure were not well documented. In other studies, alterations in human serum immunoglobulins were observed, but there was concomitant exposure to xylene and toluene (Lange et al. 1973a, 1973b). There is a need to test for subtle alterations in the immune system and immune competence in workers with intermediate- and chronic-duration exposure to benzene. Animal studies support the findings of immune dysfunction and indicate that additional parameters of the immune system are affected by exposure to benzene in the air for acute (Aoyama 1986; Chertkov et al. 1992; Cronkite 1986; Cronkite et al. 1982, 1985, 1989; Gill et al. 1980; Green et al. 1981a; Li et al. 1986; Neun et al. 1992; Rozen et al. 1984; Rosenthal and Snyder 1985; Toft et al. 1982; Ward et al. 1985; Wells and Nerland 1991), intermediate (Baarson et al. 1984; Cronkite et al. 1982, 1985, 1989; Dow 1992; Gill et al. 1980; Green et al. 1981a, 1981b; Li et al. 1992; Plappert et al. 1994b; Rosenthal and Snyder 1987; Rozen and Snyder 1985; Seidel et al. 1990; Snyder et al. 1980; Stoner et al. 1981; Ward et al. 1985; Wolf et al. 1956; Songnian et al. 1982), and chronic (Snyder et al. 1980, 1984, 1988) periods. An acute-duration inhalation study found depressions of proliferative responses of bone-marrow-derived B-cells and splenic T-cells in mice at 10 ppm (Rozen et al. 1984), and an acute-duration inhalation MRL has been derived based on this study. An intermediate-duration inhalation study found delayed splenic lymphocyte reaction to foreign antigens evaluated by *in vitro* mixed lymphocyte culture following exposure of mice to benzene vapors at a concentration of 10 ppm (Rosenthal and Snyder 1987), and an intermediate-duration inhalation MRL has been derived based on this study. No human data on the oral route were available, but animal data showed immunological

effects after intermediate (Fan 1992; Hsieh et al. 1988b, 1990, 1991; Shell 1992; Wolf et al. 1956) and chronic exposures (Huff et al. 1989; Maltoni et al. 1983, 1985; NTP 1986) via the oral route. Since the immune system is known to be a target organ, it is important to have information on subtle alterations in immune competence in people exposed to benzene in the air and in the drinking water. A decrease in cell-mediated immune functions, including alloantigen response and cytotoxicity, was reported in mice following intermediate inhalation exposure to 100 ppm of benzene (Rosenthal and Snyder 1987). This impaired cell-mediated immune function was also apparent in other *in vivo* studies (Stoner et al. 1981). Mice exposed to 100 ppm of benzene for a total of 100 days had reduced tumor resistance when challenged with syngeneic tumor cells and developed tumors that were lethal (Rosenthal and Snyder 1987). Further animal studies would also be useful in defining more NOAEL and LOAEL values. No data are available that document immunotoxicity in humans or animals exposed by dermal application. Dermal sensitization tests may also provide useful data on the likelihood of an allergic response in humans, since skin contact may occur in the workplace and at hazardous waste sites.

Neurotoxicity. In humans, the nervous system is a target of benzene toxicity following both inhalation and oral exposures. No data are available that demonstrate neurologic effects in humans or animals exposed dermally. There are sufficient data to suggest that it is the central nervous system which is affected following acute exposures. Neurological symptoms reported in humans following acute oral and inhalation exposures are similar and include drowsiness, dizziness, headache, vertigo, tremor, delirium, and loss of consciousness (Cronin 1924; Flury 1928; Greenburg 1926; Midzenski et al. 1992; Tauber 1970; Thienes and Haley 1972). Acute- and intermediate-duration inhalation and oral animal studies provide supportive evidence that benzene effects the central nervous system (Carpenter et al. 1944; Cornish and Ryan 1965; Evans et al. 1981; Frantik et al. 1994). Effects observed include narcosis, hyperactivity, tremors, tonic-clonic convulsions, decreased evoked electrical activity in the brain, and slight nervous system depression. Behavioral changes were found in mice after 1 week of exposure to 300 ppm, and decreased grip strength was found after three exposures to 1,000 ppm (Dempster et al. 1984). An intermediate-duration inhalation study found increased rapid response in mice at 0.78 ppm (Li et al. 1992), but this study was limited due to apparent discrepancies between reported and actual benzene exposure levels, since neurological effects reported in other animal studies occurred only at much higher exposure levels. Acute-duration oral exposures of 950 mg/kg benzene altered neuro-transmitter levels in the brains of rats (Kanada et al. 1994). Intermediate-duration oral exposures of 8 mg/kg/day resulted in changes in the levels of monoamine transmitters in the brain without treatment-related behavioral changes (Hsieh et al. 1988a).

One chronic-duration occupational study reported neurological abnormalities of the peripheral nervous system (global atrophy of lower extremities and distal neuropathy of upper extremities) in four of six benzene-exposed patients with aplastic anemia (Baslo and Aksoy 1982). Additional studies are needed to verify the peripheral nervous system effects that might occur following chronic exposures to low doses of benzene.

Although there are sufficient data to indicate that the nervous system is a target of benzene toxicity, the neurotoxicity of benzene has not been extensively studied. Additional studies in animals are needed to identify the thresholds of neurotoxicity following acute-, intermediate-, and chronic-duration inhalation and oral exposures.

Epidemiological and Human Dosimetry Studies. A large segment of the U.S. population is exposed to benzene. This exposure occurs primarily as a result of benzene emitted to the air from tobacco smoke, gasoline stations, and automobile exhaust. Benzene has been found in about a third of the NPL hazardous waste sites. The magnitude of exposure is greater for those occupationally exposed.

The predominance of available epidemiological data comes from occupational studies and associated analysis (Aksoy and Erdem 1978; Aksoy et al. 1971, 1972, 1974; Baslo and Aksoy 1982; Ciccone et al. 1993; Cody et al. 1993; Dosemeci et al. 1994, 1996; Doskin 1971; EPA 1986, 1995, 1998; Erf and Rhoads 1939; Goldwater 1941; Greenburg et al. 1939; Hayes et al. 1997; IARC 1982, 1987; Infante 1978; Infante et al. 1977; IRIS 2007; Kipen et al. 1989; Lan et al. 2004a, 2004b; Li et al. 1994; Ott et al. 1978; Paustenbach et al. 1992; Paxton et al. 1994a, 1994b; Qu et al. 2002, 2003a, 2003b; Rinsky et al. 1981, 1987, 2002; Rothman et al. 1996a, 1996b; Townsend et al. 1978; Travis et al. 1994; Utterback and Rinsky 1995; Ward et al. 1996; Yin et al. 1987c, 1989, 1994, 1996a, 1996b). While these studies provide data on hematological and neurotoxic effects and evidence of carcinogenicity, they only offer information on the effects of inhalation exposure. Retrospective and prospective studies of populations that have been identified as being exposed to contaminated groundwater or drinking water could provide information on long-term health effects from oral exposures. The Benzene Subregistry Baseline Technical Report of the National Exposure Registry contains information on 1,143 persons who had documented exposure to benzene in their drinking water and were exposed for at least 30 days (Agency for Toxic Substances and Disease Registry 2001). No causal relationship has been proposed for health conditions identified in the base subregistry (Agency for Toxic Substances and Disease Registry 1995) or continued follow-up of the population (Agency for Toxic Substances and Disease Registry 2001).

Few well-conducted epidemiological studies of exposed populations exist. An epidemiological study of a population with chronic exposure to two NPL hazardous waste sites illustrates the problems inherent in assessing adverse health effects from waste site exposure (Dayal et al. 1995). Benzene was only 1 of 20 compounds or groups of compounds identified as being chemicals of concern in the waste site. The authors note that assessment of long-term exposure to a dumpsite is problematic because of a lack of history of materials deposited, the complexity of the waste components, the interaction of the components over time, and the emphasis on statistically significant versus biologically significant effects. The study had certain limitations, including lifestyle characteristics, the use of mailed-in responses, subjective symptom-reports without independent confirmation, and various problems with exposure estimation. A relationship was detected between exposed individuals and neurological symptoms, including learning difficulties, nervousness, numbness in fingers or toes, sleeping difficulties, unusual fatigue, and decreased sense of smell. Chest pains, irregular heartbeat, skin rashes, and detection of a peculiar odor or taste also occurred more often in the exposed population. However, no effort was made to relate the symptoms to particular chemicals in the waste site. Clearly, more studies of exposed populations surrounding waste sites would be warranted. Other studies include analyses of workers in China (Dosemeci et al. 1994, 1996; Lan et al. 2004a, 2004b; Qu et al. 2002, 2003a, 2003b; Rothman et al. 1996a, 1996b; Yin et al. 1987c, 1994), reanalysis of data from the Pliofilm cohort (Paxton et al. 1994a, 1994b; Utterback and Rinsky 1995; Ward et al. 1996), and assessment of clinical manifestations of benzene hematotoxicity in U.S. industries with potential for benzene exposure (Collins et al. 1991, 1997; Tsai et al. 1983, 2004). A publication by Lioy and Pellizzari (1995) outlines an appropriate framework for the National Human Exposure Assessment Survey, using benzene as a candidate compound. Other articles also address population-based exposure models and measurements (MacIntosh et al. 1995; Pellizzari et al. 1995).

Analysis of benzene dosimetry in animal studies suggests that the metabolic pathways leading to the production of the putative toxic metabolites appear to be low-capacity, high-affinity pathways that are saturated at relatively low-exposure concentrations (Henderson et al. 1992). The authors suggest that this could also be true for humans. Additional dosimetry studies are necessary to further define the role of toxic metabolites in the development of benzene-related leukemia.

Studies of DNA damage have primarily been performed on workers with preexisting hematological effects and cancer (Ding et al. 1983; Forni et al. 1971a; Picciano 1979; Yardley-Jones et al. 1988) or as biomonitoring of benzene exposure (Andreoli et al. 1997; Bogadi-Šare et al. 1997; Hallberg et al. 1996; Kašuba et al. 2000; Liu et al. 1996; Nilsson et al. 1996; Pitarque et al. 1996; Sul et al. 2002; Surrallés et al. 1997; Zhang et al. 1999). Additional data on benzene-induced DNA damage could be useful in

monitoring possible adverse effects in individuals living near hazardous waste sites if more quantitative dose-response data were available for clastogenicity.

Studies to determine whether benzene causes solid tumors and hematological malignancies other than acute nonlymphocytic leukemia in humans have been suggested as a data need (Goldstein and Warren 1993). These data could be obtained by revisiting already established cohorts of exposed individuals, or by examining new cohorts.

Biomarkers of Exposure and Effect.

Exposure. There is no clinical disease state unique to benzene toxicity. However, the effects on the hematopoietic and immune systems are well recognized, and analytical methodologies exist for monitoring benzene levels in expired breath and blood (Brugnone et al. 1989, 1992; DeLeon and Antoine 1985; Pellizzari et al. 1988). Several biomarkers of exposure to benzene exist, including unmetabolized benzene in expired air and urine (Farmer et al. 2005; Fustinoni et al. 2005; Ghittori et al. 1993; Nomiyama and Nomiyama 1974a, 1974b; Sherwood 1988; Srbova et al. 1950; Waidyanatha et al. 2001), urinary phenol levels (Astier 1992; Inoue et al. 1986, 1988b; Jongeneelen et al. 1987; Karacic et al. 1987; Pagnotto et al. 1961; Pekari et al. 1992), and urinary *trans*,*trans*-muconic acid and *S*-phenylmercapturic acid levels (Boogaard and van Sittert 1995, 1996; Ducos et al. 1990, 1992; Farmer et al. 2005; Inoue et al. 1989b, 2000; Lee et al. 1993; Melikian et al. 1993, 1994; Pezzagno et al. 1999; Popp et al. 1994; Qu et al. 2005; Rothman et al. 1998; Ruppert et al. 1997; Sanguinetti et al. 2001; van Sittert et al. 1993; Weaver et al. 2000). Estimates of benzene exposure can be made by comparing the ratio of inorganic to organic sulfates in the urine (Hammond and Hermann 1960). However, urinary sulfate levels are variable, and they have not been used to identify exposure levels of benzene associated with minimal toxic effect. Furthermore, benzene-derived adducts with hemoglobin and albumin may be useful as biomarkers (Bechtold et al. 1992a, 1992b; Bechtold and Henderson 1993; Farmer et al. 2005; Fustinoni et al. 2005; Ghittori et al. 1993; Hedli et al. 1991; Lutz and Schlatter 1977; Nomiyama and Nomiyama 1974a, 1974b; Pekari et al. 1992; Reddy et al. 1989; Schad et al. 1992; Sherwood 1988; Smith and Rothman 2000; Srbova et al. 1950; Waidyanatha et al. 2001; Yeowell-O'Connell et al. 1998, 2001).

Phenol measurements have routinely been used for monitoring occupational exposures, and there is evidence that urinary phenol levels can be correlated with exposure levels (Astier 1992; Inoue et al. 1986; Pekari et al. 1992). Correlating urinary phenol with benzene exposure is complicated by high and variable background levels in nonexposed persons. The data suggest that variations in urinary phenol will

obscure phenol formed from low levels of benzene (Ong and Lee 1994; Perbellini et al. 1988). For exposures to 1 ppm or less, the current workplace standard, urinary phenol is not an adequate assay to determine the extent of benzene exposure. In addition, urinary excretion of phenol is lowered by coexposure to toluene (Inoue et al. 1988b). In many exposure situations (e.g., occupational settings and hazardous waste sites), toluene and other chemicals are present and could interfere with the metabolism and elimination of benzene. Studies that attempt to identify another biomarker that is specific to benzene, such as *S*-phenylmercapturic acid, the proposed urinary BEI (ACGIH 1996b), may be helpful for medical surveillance.

The metabolism of benzene to ring-opened compounds (e.g., muconic acid) may be pertinent to the development of a biomarker of benzene exposure and effect (Ducos et al. 1990, 1992; Inoue et al. 1989b; Lee et al. 1993; Melikian et al. 1993; Ong and Lee 1994; Witz et al. 1990b). However, the dose-response curve for metabolism of benzene to urinary muconic acid in humans needs to be determined. If urinary muconic acid increases in humans at low benzene exposures, as it does in mice, then it may be a valid biomarker of benzene exposure. Excretion of urinary muconic acid in humans has also been shown to be lowered by coexposure to toluene (Inoue et al. 1989b). The effect of coexposure to toluene and other chemicals on muconic acid formation needs to be assessed in order to determine the usefulness of muconic acid as a biomarker of benzene exposure.

Breath levels of benzene have been used as a measure of exposure (Brugnone et al. 1989; Money and Gray 1989; Nomiyama and Nomiyama 1974a, 1974b; Ong and Lee 1994; Pekari et al. 1992). However, the amount of benzene lost in expired air will vary not only with the dose, duration, and time from exposure, but also with the extent of metabolism in the body. Benzene levels in blood have been measured. However, blood levels rapidly decrease after exposure (Brugnone et al. 1989; DeLeon and Antoine 1985; Schrenk et al. 1941).

Benzene metabolites also form DNA adducts (Popp et al. 1992). The use of hemoglobin adducts formed from benzene metabolites as a biomarker of benzene exposure has been developed by Sun et al. (1990). Bechtold and colleagues have conducted further studies using this model (Bechtold et al. 1992a; Bechtold and Henderson 1993). Further refinement of this model would be useful to quantitate cumulative low-level exposures to benzene. Development of specific markers of exposure to use in epidemiological studies to clarify the shape of the dose-response curve in the low-dose region has been suggested as a data need (Goldstein and Warren 1993). Recent reports indicate that urinary benzene may serve as a sensitive

biomarker of exposure to benzene concentrations well below 1 ppm (Farmer et al. 2005; Fustinoni et al. 2005).

Effect. Monitoring of benzene workers has included monthly blood counts. Toxic effects occur in the bone marrow and arise either from benzene via a solvent effect, or via its metabolites (Gad-El-Karim et al. 1985; Irons et al. 1980). Hematological tests could be used as markers of hematotoxicity, but medical laboratories lack tests specific to benzene hematotoxicity. The tests cannot be relied upon to find preclinical disease but can identify the subtle changes that are early indicators of effects. Furthermore, these markers of effect may not be useful for long periods following cessation of exposure, nor do they distinguish between acute and chronic exposures. As stated above, additional studies are needed to define the role of benzene-related leukopenia in the disease process initiated by benzene exposure, to determine if it is a biomarker of effect, or an intermediate end point in the development of leukemia (Hayes 1992).

Absorption, Distribution, Metabolism, and Excretion. Data from both humans and animals consistently indicate that benzene is rapidly absorbed through the lungs (Eutermoser et al. 1986; Nomiyama and Nomiyama 1974a; Sabourin et al. 1987; Schrenk et al. 1941; Srbova et al. 1950; Yu and Weisel 1996). Although experimentally-acquired data are not available on oral absorption of benzene in humans, case reports of accidental or intentional poisoning suggest that benzene is rapidly absorbed from the gastrointestinal tract (Thienes and Haley 1972). The efficient absorption of oral doses in animals is well documented (Cornish and Ryan 1965; Parke and Williams 1953a; Sabourin et al. 1987). Benzene can be absorbed through the skin, but the rate of absorption is much lower than that for inhalation (Maibach and Anjo 1981; Susten et al. 1985; Tsuruta 1989). Following absorption into the body, benzene is widely distributed to tissues, with the relative uptake dependent on the perfusion of the tissue by blood, and the total potential uptake dependent on fat content and metabolism (Sato et al. 1975; Tauber 1970).

There is no evidence to suggest that the route of administration has any substantial effect on the subsequent metabolism of benzene, either in humans or animals. Benzene is metabolized primarily in the liver and to a lesser extent, in the bone marrow. Benzene is a preferential substrate of CYP2E1, which also metabolizes alcohol. The induction of CYP2E1 by benzene (and some of its metabolites) with subsequent generation of reactive metabolites, oxygen radicals, circulating lipid peroxides, and hydroxyl radicals could be associated with hematopoietic toxicity and carcinogenicity of benzene (Irons 2000; Parke 1989; Ross 1996, 2000; Smith 1996a, 1996b; Snyder 2000a, 2000b, 2002; Snyder and Hedli 1996; Snyder and Kalf 1994). CYP2E1 is not confined to the liver, but has also been detected in bone marrow. Andrews et al. (1979) demonstrated that rabbit bone marrow is capable of metabolizing benzene. Schnier

et al. (1989) subsequently found that rabbit bone marrow contains CYP2E1. Irons et al. (1980) demonstrated that benzene metabolism by rat bone marrow (*in situ*) was complete and independent of metabolism by the liver, with concentrations of phenol greater than catechol and hydroquinone. Although the total metabolism by bone marrow was limited (total metabolites present were 25% of those in blood), the concentration of metabolites in the bone marrow exceeded that in the blood. Similar studies have been conducted in mice (Ganousis et al. 1992). Benzene metabolism in bone marrow is not well understood; additional data regarding the initial oxidation step and the comparatively low levels of CYP2E1 activity in bone marrow would be useful in identifying the mechanisms of benzene's hematotoxicity. This aspect of metabolism may have implications for long-term exposures, which could be explored in chronic exposure studies. The intermediary metabolites of benzene are responsible for many of the toxic effects observed (Eastmond et al. 1987; Gad-El-Karim et al. 1985). Biotransformation is believed to be essential for benzene-induced bone marrow damage.

However, there is disagreement as to whether benzene is activated in the marrow, activated elsewhere and transported to the marrow, or metabolized in the liver and the metabolites activated in the marrow. Further studies on the metabolism of benzene would help define its mechanism of action. Additionally, more information is needed on the pathways of metabolism in humans, the chemical nature of the toxic metabolites, and the mechanism of toxicity. Recently published data comparing urinary metabolite profiles of orally administered benzene and phenol in mice suggest that zonal differences in metabolism in the liver may be responsible for relative differences in the production of hydroquinone, thus explaining the higher toxicity observed after benzene administration compared with phenol administration (Kenyon et al. 1995). Additional work in this area would aid in further understanding the kinetic determinants of benzene toxicity. Ethanol and dietary factors such as food deprivation and carbohydrate restriction enhance the hematotoxic effects of benzene. Therefore, more information regarding differences in metabolic pattern according to sex, age, nutritional status, and species, and correlation to differences in health effects would be useful.

Humans and animals both excrete inhaled benzene via expiration. Additionally, benzene metabolites are excreted primarily in the urine in both humans and animals. No studies in humans exist for excretion of oral doses of benzene. Studies in several animal species indicate that the route of excretion of benzene and/or its metabolites is a function of exposure level and the saturation of metabolic systems (Henderson et al. 1989). Data regarding excretion following dermal exposure in humans are limited. However, the major route of excretion in both humans and animals following dermal exposure is the urine.

Comparative Toxicokinetics. Qualitatively, absorption, distribution, metabolism, and excretion appear to be similar in humans and laboratory animals. However, quantitative variations in the absorption, distribution, metabolism, and excretion of benzene have been observed with respect to exposure routes, sex, nutritional status, and species. Further studies that focus on these differences and their implications for human health would be useful. Additionally, *in vitro* studies using human tissue and further research into PBPK modeling in animals would contribute significantly to the understanding of the kinetics of benzene and would aid in the development of pharmacokinetic models of exposure in humans. These topics are being addressed in ongoing studies (see Section 3.12.3).

Methods for Reducing Toxic Effects. Development of methods and practices that are specific for benzene is needed for reducing peak absorption, body burden and for interfering with the mechanism of action following benzene exposures. Since benzene metabolites are thought to play the major role in the toxicity and carcinogenicity, more information is needed about their covalent binding to nucleic acids and cellular macromolecules. This information would help the development of methods for possible prevention of benzene-induced toxicity. Related lines of investigation include the use of non-steroidal anti-inflammatory drugs to block prostaglandin and prostaglandin synthetase-mediated activity after benzene exposure, and the role of IL-1 cytokine activity in preventing depression of hematopoiesis.

Children's Susceptibility. Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection above.

No clear evidence of age-related differences in susceptibility to benzene toxicity was located. Benzene crosses the placenta (Dowty et al. 1976). Nursing infants can be exposed to benzene in the breast milk (Fabietti et al. 2004). Limited animal studies indicate that *in utero* exposure to benzene results in hematological changes similar to those observed in animals exposed only as adults (Corti and Snyder 1996; Keller and Snyder 1986, 1988). There is some indication that parental occupational exposure to benzene may play a role in childhood leukemia (Buckley et al. 1989; McKinney et al. 1991; Shaw et al. 1984; Shu et al. 1988). However, none of these studies indicate whether children may be at greater risk than adults for benzene toxicity. Children could potentially be at increased risk for benzene toxicity via the inhalation exposure route based on higher activity levels and ventilation rates than adults. Age-related differences in benzene metabolism could potentially affect susceptibility. Well-designed animal studies should be performed to adequately assess the potential for age-related increased susceptibility to benzene.

Child health data needs relating to exposure are discussed in Section 6.8.1, Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

Ongoing studies pertaining to benzene have been identified and are shown in Table 3-7 (FEDRIP 2005).

Table 3-7. Ongoing Studies on the Health Effects of Benzene

Investigator	Affiliation	Research description	Sponsor
Conaway CC	University of California Lawrence Livermore National Laboratory	DNA binding of radiolabeled benzene	National Center for Research Resources
French JE	National Institute of Environmental Health Sciences	Carcinogen inactivation of tumor suppressor genes in p53 mice	National Institute of Environmental Health Sciences
French JE	National Institute of Environmental Health Sciences	Mechanisms of leukemogenesis in genetically-altered mice	National Institute of Environmental Health Sciences
Sabri MI	Oregon Health and Science University	Biomarkers of exposure and effect in neurotoxicants including neurotic benzene derivatives	National Institute of Environmental Health Sciences
Monks TJ	University of Texas at Austin	Benzene metabolites and hematotoxicity	National Institute of Environmental Health Sciences
Ross D	Texas A&M University System	Protective effect of NQ01 against benzene toxicity	National Institute of Environmental Health Sciences
Smith MT	University of California Berkeley	Biomarkers of benzene exposure and genotoxicity	National Institute of Environmental Health Sciences

Source: FEDRIP 2005

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4. CHEMICAL AND PHYSICAL INFORMATION

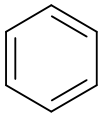
4.1 CHEMICAL IDENTITY

Information regarding the chemical identity of benzene is located in Table 4-1. Although the term benzol is found in older literature for the commercial product, benzene is the name presently approved by the International Union of Pure and Applied Chemistry (IUPAC), the Chemical Manufacturers Association (CMA), and the American Society for Testing and Materials (ASTM) for the pure product.

4.2 PHYSICAL AND CHEMICAL PROPERTIES

Information regarding the physical and chemical properties of benzene is located in Table 4-2. The major impurities found in commercial products are toluene, xylene, phenol, thiophene, carbon disulfide, acetylnitrile, and pyridine (NIOSH 1974). Commercial refined benzene-535 is free of hydrogen sulfide and sulfur dioxide, but contains a maximum of 1 ppm thiophene and a maximum of 0.15% nonaromatics. Refined nitration-grade benzene is free of hydrogen sulfide and sulfur dioxide. Benzene is also commercially available as thiophene-free, 99 mole%, 99.94 mole%, and nanograde quality (HSDB 2007).

Table 4-1. Chemical Identity of Benzene

Characteristic	Information	Reference
Chemical name	Benzene	HSDB 2007
Synonym(s)	Annulene, benzeen (Dutch), benzen (Polish), benzol, benzole; benzolo (Italian), coal naphtha, cyclohexatriene, fenzen (Czech), phene, phenyl hydride, pyrobenzol, pyrobenzole	HSDB 2007
Registered trade name(s)	Polystream	IARC 1982
Chemical formula	C_6H_6	Budavari et al. 2001
Chemical structure		Budavari et al. 2001
Identification numbers:		
CAS registry	71-43-2	HSDB 2007
NIOSH RTECS	CY-1400000	HSDB 2007
EPA hazardous waste	NA	
OHM/TADS	No Data	
DOT/UN/NA/IMCO shipping	UN1114; IMO3.2	HSDB 2007
HSDB	35	HSDB 2007
NCI	C55276	HSDB 2007
Merck	1066	Budavari et al. 2001

CAS = Chemical Abstracts Service; DOT/UN/NA/IMO=Department of Transportation/United Nations/North America/ Intergovernmental Maritime Dangerous Goods Code; EPA = Environmental Protection Agency; HSDB=Hazardous Substances Data Bank; NCI = National Cancer Institute; NIOSH= National Institute for Occupational Safety and Health; OHM/TADS = Oil and Hazardous Materials/Technical Assistance Data System; RTECS = Registry of Toxic Effects of Chemical Substances

Table 4-2. Physical and Chemical Properties of Benzene

Property	Information	Reference
Molecular weight	78.11	Budavari et al. 2001
Color	Clear, colorless liquid	Budavari et al. 2001
Physical state	colorless to light yellow liquid	HSDB 2007
Melting point	5.5 °C	Budavari et al. 2001
Boiling point	80.1 °C	Budavari et al. 2001
Density at 15 °C, g/cm^3	0.8787	Budavari et al. 2001
Odor	Aromatic	NFPA 1994
Odor threshold:		
Water	2.0 mg/L	HSDB 2007
Air[a]	Detection range: 34–119 ppm (geometric mean: 61 ppm) Recognition: 97 ppm	AIHA 1989
Taste threshold:	0.5–4.5 mg/L	HSDB 2007
Solubility:		
Water at 25 °C	w/w: 0.188%	Budavari et al. 2001
Organic solvents	Alcohol, chloroform, ether, carbon disulfide, acetone, oils, carbon, tetrachloride, glacial acetic acid	Budavari et al. 2001
Partition coefficients:		HSDB 2007; Karickhoff 1981; Kenaga 1980
Log K_{ow}[b]	2.13	
Log K_{oc}[c]	1.8–1.9	
Vapor pressure at 20 °C	75 mm Hg	NFPA 1994
Henry's law constant at 25 °C	5.5×10^{-3} atm-m^3/mol	Mackay and Leinonen 1975
Autoignition temperature	498 °C	NFPA 1994
Flashpoint	-11 °C (closed cup)	Budavari et al. 2001
NFPA hazard classification:		HSDB 2007
Health	2.2	
Flammability	3.3	
Reactivity	0.0	
Flammability limits in air	1.2% (lower limit; 7.8% (upper limit)	NFPA 1994
Conversion factors	1 ppm=3.26 mg/m^3 at 20 °C and 1 atm pressure; 1 mg/m^3=0.31 ppm	HSDB 2007
Explosive limits	1.4% (lower limit); 8% (upper limit)	HSDB 2007

[a]Odor threshold values considered by AIHA (1989) to be acceptable based on review of peer-reviewed reports of odor thresholds for benzene (range 0.78–100 ppm).
[b]K_{ow} = octanol-water partitioning coefficient
[c]K_{oc} = soil adsorption coefficient

NFPA = National Fire Protection Association

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5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

5.1 PRODUCTION

In 1825, Faraday isolated benzene from a liquid condensed by compressing oil gas. Benzene was first synthesized by Mitscherlich in 1833 by distilling benzoic acid with lime. Benzene was first commercially recovered from light oil derived from coal tar in 1849 and from petroleum in 1941 (IARC 1982a). Several years after the end of World War II, the rapidly expanding chemical industry created an increased demand for benzene that the coal carbonization industry could not fulfill. To meet this demand, benzene was produced by the petroleum and petrochemical industries by recovery from reformat and liquid byproducts of the ethylene manufacturing process (Fruscella 1992).

Currently, benzene is commercially recovered from both coal and petroleum sources. More than 98% of the benzene produced in the United States is derived from the petrochemical and petroleum refining industries (OSHA 1987). These sources include refinery streams (catalytic reformats), pyrolysis gasoline, and toluene hydrodealkylation. Catalytic reformat is the major source of benzene (Greek 1990). Between 1978 and 1981, catalytic reformats accounted for approximately 44–50% of the total U.S. benzene production (Fishbein 1988). During catalytic reforming, cycloparaffins (also known by the obsolescent term "naphthenes") such as cyclohexane, methyl cyclohexane, and dimethylcyclohexane are converted to benzene by isomerization, dehydrogenation, and dealkylation, and paraffins in naphtha (such as hexane) are converted to benzene by cyclodehydrogenation. The process conditions and the catalyst determine which reaction will predominate. The benzene is recovered by solvent extraction (e.g., with sulpholane or tetraethylene glycol). Pyrolysis gasoline is a liquid byproduct produced by the steam cracking of lower paraffins (gas oil) or heavier hydrocarbons (heavy naphtha). Pyrolysis gasoline contains unsaturated aliphatic hydrocarbons (such as ethylene and propylene) and aromatics. Several integrated pyrolysis gasoline treatment processes are available including partial hydrogenation and extractive distillation; hydrogenation, hydrodesulfurization, and solvent extraction; or partial hydrogenation, desulfurization, hydrocracking, hydrodealkylation, and distillation for the optimization of benzene yield and the recovery of benzene (Fruscella 1992; IARC 1982a). In the toluene hydrodealkylation process, toluene or toluene/xylene mixtures are reacted with hydrogen at temperatures ranging from 500 to 595 °C with usual pressures of 4–6 mPa (40–60 atm), and demethylated to produce benzene and methane. Another process whereby toluene is converted to benzene and xylenes by transalkylation or disproportionation is also used for the production of benzene (Fruscella 1992). Small quantities of benzene are also produced from destructive distillation of coal used for coke manufacture. Benzene is derived from the light oil fraction produced during the coking process (Greek 1990; Fruscella 1992). New coking, gasification, and

liquefaction processes for coal are all potential sources of benzene (IARC 1982a). Of the total U.S. production capacity of 3.109 billion gallons in 2004, catalytic reformats constituted 45%, toluene and xylene 30%, pyrolysis gasoline 23%, and coke oven <2% (SRI 2004).

Benzene ranks in the top 20 most abundantly produced chemicals in the United States (C&EN 1994; Kirschner 1995; Reisch 1994). Production data from 1984 to 1994 indicate that the production of benzene increased by about 4% annually (C&EN 1995).

According to the Toxics Release Inventory (TRI), 2,528 facilities in the United States produced or processed benzene in 2004 (TRI04 2006). Table 5-1 lists the facilities in each state that manufacture or process benzene, the intended use, and the range of maximum amounts of benzene that are stored on site. The TRI data listed in Table 5-1 should be used with caution since only certain types of facilities were required to report. Therefore, this is not an exhaustive list.

SRI (2004) lists the companies producing benzene in the United States in 2004. This list is summarized in Table 5-2. Facilities listed as being responsible for producing the top 50% of regional capacity in the United States include: Exxon Mobil Chemical Company, 518 million gallons (16%); Equistar Chemicals LP, 330 million gallons (11%), Dow Chemical Company, 300 million gallons (10%); Flint Hills Resources LP, 250 million gallons (8%), and BP Oil Company 230 million gallons (7%) (SRI 2004).

5.2 IMPORT/EXPORT

Benzene is imported and exported to the United States as both the pure chemical and as a mixture of mineral fuels. The import of pure benzene into the United States is dependent on domestic production and demand. Imports of benzene (from mineral fuels and pure benzene) into the United States were approximately 5,252 million L (10,176 million pounds) in 2004, 6,024 million L (11,672 million pounds) in 2003, and 5,912 million L (11,455 million pounds) in 2002 (USITC 2005). The largest exporters of benzene to the United States during 2002-2004 were Canada, Iraq, Israel, Kuwait, Venezuela, and Saudi Arabia (USITC 2005).

As in the case of import, the export of benzene from the United States to other countries is dependent on domestic and world production and demand. The 2002–2004 data indicate an increase in export volumes for benzene during this period. Exports of benzene (both pure benzene and benzene derived from mineral fuels) to other countries were approximately 11 million L (21 million pounds) in 2002, 150 million L

Table 5-1. Facilities that Produce, Process, or Use Benzene

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
AK	26	0	99,999,999	1, 2, 3, 4, 5, 7, 8, 9, 11, 12
AL	85	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
AR	24	100	9,999,999	1, 2, 3, 4, 6, 8, 9, 11, 12, 13
AS	1	100,000	999,999	9
AZ	30	100	9,999,999	1, 2, 4, 5, 7, 9, 10, 11, 12, 13, 14
CA	172	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14
CO	23	0	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 13
CT	8	1,000	9,999,999	1, 4, 6, 7, 9, 10, 12
DE	13	1,000	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13
FL	36	0	499,999,999	1, 2, 3, 4, 5, 7, 8, 9, 10, 12, 13, 14
GA	33	0	49,999,999	1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
GU	6	100	9,999,999	2, 3, 4, 7, 9, 12
HI	15	100	49,999,999	1, 2, 3, 4, 5, 6, 7, 9, 12, 13, 14
IA	21	0	10,000,000,000	1, 2, 3, 4, 5, 7, 9, 10, 11, 12, 13, 14
ID	3	10,000	9,999,999	7, 9, 12
IL	102	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
IN	80	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
KS	59	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14
KY	66	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
LA	212	0	10,000,000,000	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
MA	15	100	99,999,999	2, 4, 7, 8, 9
MD	20	100	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12
ME	9	100	9,999,999	2, 3, 4, 7, 9, 12, 13
MI	108	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
MN	36	100	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
MO	30	0	9,999,999	1, 5, 7, 8, 9, 11, 12
MP	7	0	999,999	2, 3, 4, 7, 9
MS	43	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14
MT	27	10,000	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 13, 14
NC	34	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13
ND	9	0	49,999,999	1, 2, 3, 4, 5, 6, 9, 12, 13
NE	11	0	9,999,999	1, 7, 10, 12, 13
NH	9	0	999,999	1, 2, 7, 9, 12, 13
NJ	61	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13
NM	32	100	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 13, 14
NV	5	100	99,999	1, 2, 4, 8, 9, 12, 13
NY	48	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13
OH	101	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14

Table 5-1. Facilities that Produce, Process, or Use Benzene

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
OK	48	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14
OR	12	1,000	9,999,999	1, 2, 3, 4, 5, 7, 9, 12, 13
PA	100	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
PR	52	0	10,000,000,000	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13
RI	9	10,000	9,999,999	1, 2, 4, 7, 8, 9
SC	26	0	9,999,999	1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13
SD	8	100	999,999	2, 3, 4, 7, 8, 11, 12, 13, 14
TN	53	0	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
TX	361	0	10,000,000,000	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
UT	42	1,000	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13
VA	36	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13
VI	8	10,000	499,999,999	1, 2, 3, 4, 6, 7, 8, 9
WA	57	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 13, 14
WI	27	0	9,999,999	1, 4, 5, 7, 8, 9, 11, 12, 13, 14
WV	32	0	99,999,999	1, 2, 4, 5, 6, 7, 8, 9, 12, 13
WY	37	0	9,999,999	1, 2, 3, 4, 5, 6, 7, 9, 12, 13, 14

[a]Post office state abbreviations used
[b]Amounts on site reported by facilities in each state
[c]Activities/Uses:

1. Produce
2. Import
3. Onsite use/processing
4. Sale/Distribution
5. Byproduct
6. Impurity
7. Reactant
8. Formulation Component
9. Article Component
10. Repackaging
11. Chemical Processing Aid
12. Manufacturing Aid
13. Ancillary/Other Uses
14. Process Impurity

Source: TRI04 2006 (Data are from 2004)

Table 5-2. Current U.S. Manufacturers of Benzene[a]

Company	Location (annual capacity millions of gallons)
ATOFINA Petrochemicals, Inc.	Port Arthur, Texas (87)
BASF FINA Petrochemicals, Inc.	Port Arthur, Texas (34)
BP America, Inc.	Texas City, Texas (230)
Ceveron Phillips Chemical Company, LP	Pascagoula, Mississippi (150)
Olefins and Polyolefins Business Unit	Port Arthur, Texas (35)
CITGO Petroleum Corporation	Corpus Christi, Texas (78)
	Lake Charles, Louisiana (55)
	Lemont, Illinois (19)
Conoco Phillips Refining Marketing and Transportation Division	Alliance Louisiana (65)
	Sweeny, Texas (39)
	Wood River Illinois (60)
Dow Chemical USA	Freeport, Texas (80)
	Plaquemine, Louisiana (317)
Equistar Chemicals, LP	Alvin, Texas (137)
	Channelveiw, Texas (90)
	Corpus Christi, Texas (103)
ExxonMobil Chemical Company	Baton Rouge Louisiana (100)
	Baytown, Texas (180)
	Beaumont, Texas (182)
	Chalmette, Louisiana (56)
Flint Hills Resources, LP	Corpus Christi, Texas (250)
Frontier El Dorado Refining Company	El Dorado, Kansas (15)
HOVENSA, LLC	St. Croix, Virgin Islands (60)
Huntsman, LLC	Port Arthur, Texas (90)
Lyondell-Citgo Refining, LP	Houston, Texas (50)
Marathon Ashland Petroleum, LLC	Catlettsburg, Kentucky (58)
	Texas City, Texas (3)
Motiva Enterprises, LLC	Delaware City, Delaware (15)
NOVA Chemicals Corp.	Bayport, Texas (15)
The Premcor Refining Group Inc.	Lima, Ohio (145)
Shell Chemical Company	Deer Park, Texas (180)
Sunoco, Inc.	Marcus Hook, Pennsylvania (60)
	Philadelphia, Pennsylvania (24)
	Toledo Ohio (20)
	Westville, New Jersey (18)
Valero Energy Corporation	Corpus Christi, Texas (82)
	Three Rivers, Texas (20)
Eagle-Pitcher Industries, Inc.	Lenexa, Kansas
Eagle-Pitcher Technologies, LLC	
Chem Syn Laboratiories (^{14}C-benzene)	

[a]Derived from Stanford Research Institute (SRI) 2004, receipt where otherwise noted. SRI reports production of chemicals produced in commercial quantities (defined as exceeding 5,000 pounds or $10,000 in value annually) by the companies listed.

(290 million pounds) in 2003, and 75 million L (145 million pounds) in 2004. These numbers are up from 15 million L (29 million pounds) in 2001 and 3.3 million L (6.4 million pounds) in 1993 (USITC 2005). The largest importers of benzene from the United States are Canada, the Netherlands, Taiwan, Spain, and Korea (USITC 2005).

5.3 USE

Benzene has been used extensively as a solvent in the chemical and drug industries, as a starting material and intermediate in the synthesis of numerous chemicals, and as a gasoline additive (NTP 1994).

Benzene recovered from petroleum and coal sources is used primarily as an intermediate in the manufacture of other chemicals and end products. The major uses of benzene are in the production of ethylbenzene, cumene, and cyclohexane. Ethylbenzene (55% of benzene production volume) is an intermediate in the synthesis of styrene, which is used to make plastics and elastomers. Cumene (24%) is used to produce phenol and acetone. Phenols are used in the manufacture of phenolic resins and nylon intermediates; acetone is used as a solvent and in the manufacture of pharmaceuticals. Cyclohexane (12%) is used to make nylon resins. Other industrial chemicals manufactured from benzene include nitrobenzene (5%), which is used in the production of aniline, urethanes, linear alkylbenzene sulfonates, chlorobenzene, and maleic anhydride (Eveleth 1990; Greek 1990; HSDB 2007). Benzene is also a component of gasoline since it occurs naturally in crude oil and since it is a byproduct of oil refining processes (Brief et al. 1980; Holmberg and Lundberg 1985). Benzene is especially important for unleaded gasoline because of its anti-knock characteristics. For this reason, the concentration of aromatics, such as benzene, in unleaded fuels has increased (Brief et al. 1980). The percentage by volume of benzene in unleaded gasoline is approximately 1–2% (NESCAUM 1989).

The EPA has listed benzene as a hazardous air pollutant and a hazardous waste (EPA 1977, 1981). In addition, there is sufficient evidence to support classifying benzene as a human carcinogen (Group A) (IRIS 2007). One result of EPA's action is that the widespread use of benzene as a solvent has decreased in recent years. Many products that used benzene as solvents in the past have replaced it with other organic solvents; however, benzene may still occur as a trace impurity in these products. Less than 2% of the amount produced is used as a solvent in products such as trade and industrial paints, rubber cements, adhesives, paint removers, artificial leather, and rubber goods. Benzene has also been used in the shoe manufacturing and rotogravure printing industries (EPA 1978; OSHA 1977). In the past, certain consumer products (such as some paint strippers, carburetor cleaners, denatured alcohol, and rubber

cement used in tire patch kits and arts and crafts supplies) contained small amounts of benzene (Young et al. 1978). Other consumer products that contained benzene were certain types of carpet glue, textured carpet liquid detergent, and furniture wax (Wallace et al. 1987).

The Consumer Products Safety Commission (CPSC) withdrew an earlier proposal to ban consumer products, except gasoline and laboratory reagents, that contained benzene as an intentional ingredient or as a contaminant at >0.1% by volume. The withdrawal of the rulemaking was based on CPSC findings that benzene was no longer used as an intentional ingredient and that the contaminant levels remaining in certain consumer products were unlikely to result in significant exposures (NTP 1994). Products containing >5% benzene, and paint solvents and thinners containing <10% of petroleum distillates such as benzene, are required to meet established labeling requirements. In a guidance document targeting school science laboratories, the CPSC recommended that benzene not be used or stored in schools. The document identified benzene as a carcinogen and ascertained that the hazards posed by its use in high school laboratories may be greater than its potential usefulness.

The U.S. Food and Drug Administration (FDA) regulates benzene as an indirect food additive under the Food, Drug, and Cosmetics Act (FDCA). Under the FDCA, benzene is restricted to use only as a component of adhesives used on articles intended for packaging, transport, or holding foods (FDA 1977).

5.4 DISPOSAL

Benzene-containing wastes, such as commercial chemical products, manufacturing chemical intermediates, and spent solvents, are subject to federal and/or state hazardous waste regulations (HSDB 2007). Regulations governing the treatment and disposal of benzene-containing wastes are presented in Chapter 8. Waste byproducts from benzene production processes include acid and alkali sludges, liquid-solid slurries, and solids (EPA 1982b; Saxton and Narkus-Kramer 1975). In the past, landfilling and lagooning have been the major methods of disposal of benzene-containing industrial wastes (EPA 1982b). In addition to biodegradation, a portion of the benzene is expected to be lost due to volatilization. Unfortunately benzene, along with other hazardous contaminants, also leaches into groundwater from the lagooned wastes. Currently, the recommended method of disposal is to incinerate solvent mixtures and sludges at a temperature that ensures complete combustion. The recommended methods for combustion are liquid injection incineration at a temperature range of 650–1,600 °C and a residence time of 0.1–2 seconds; rotary kiln incineration at a temperature range of 820–1,600 °C and residence times of seconds for liquids and gases, and hours for solids; and fluidized bed incineration at a temperature range of 450–

980 °C and residence times of seconds for liquids and gases and longer for solids (HSDB 2007). Since benzene burns with a very smoky flame, dilution with alcohol or acetone is suggested to minimize smoke. Small quantities of benzene waste can be destroyed by chemical reaction. For example, treating benzene with dichromate in strong sulfuric acid for 1–2 days is sufficient for total destruction (HSDB 2007).

Underground injection also appears to be an important disposal method in some states. Approximately 436,000 pounds of benzene (6% of the total environmental release) was disposed of by underground injection. This disposal via underground injection in 2006 was higher than the amount (356,000 pounds) released in 1992 (TRI90 1992), but lower than the release in 1990 (654,000 pounds) (TRI90 1992) and 2002 (692,000 pounds) (TRI02 2005). In addition, 24,000 pounds of benzene (0.3% of the total environmental release) was disposed of via land disposal (TRI04 2006). The amount discharged to soil in 2002 was less than one quarter of the amount (724,000 pounds) discharged in 1990 (TRI90 1992) and less than one third of the amount (340,000 pounds) discharged in 1992 (TRI92 1994).

Several methods exist for the treatment of waste water that contains benzene: biological treatment (aeration or activated sludge process), solvent extraction, air and/or steam stripping, and activated carbon process (EPA 1994a; IRPTC 1985). Full-scale chemical treatability studies have demonstrated 95–100% reductions in benzene concentrations for industrial waste waters receiving biological treatments (HSDB 2007). A combination of steam stripping and air stripping, and a vapor extraction system that removes the separated benzene vapor may be suitable for the treatment of contaminated groundwater and soil (Naft 1992). An *in situ* bioremediation process has been used to decontaminate a site by delivering a controlled amount of nitrate (to accelerate biodegradation of benzene) to the site under hydraulic control (Kennedy and Hutchins 1992).

6. POTENTIAL FOR HUMAN EXPOSURE

6.1 OVERVIEW

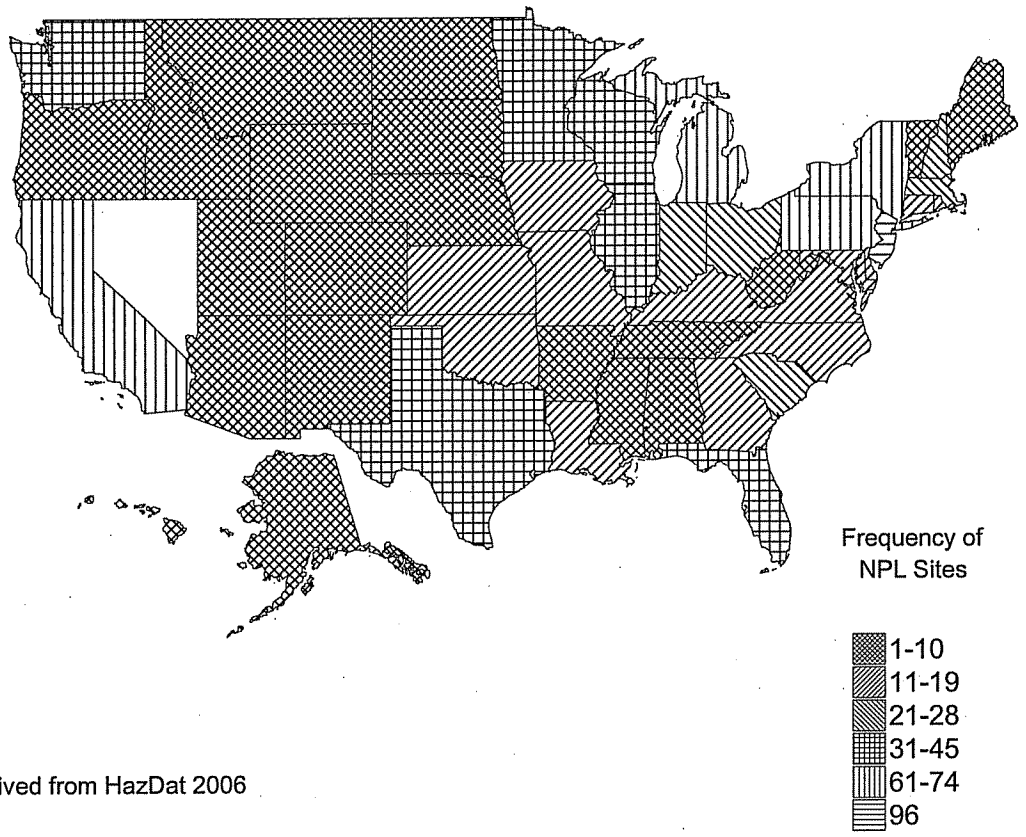
Benzene has been identified in at least 1,000 of the 1,684 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (HazDat 2006). However, the number of sites evaluated for benzene is not known. The frequency of these sites can be seen in Figure 6-1. Of these sites, 995 are located within the United States, 3 are located in the Commonwealth of Puerto Rico (not shown), and 2 are located in the Virgin Islands (not shown).

Benzene is released to the environment by both natural and industrial sources, although the anthropogenic emissions are undoubtedly the most important. Emissions of benzene to the atmosphere result from gasoline vapors, auto exhaust, and chemical production and user facilities. EPA's estimate of nationwide benzene atmospheric emissions from various sources was 34,000 metric tons/year (EPA 1989). According to the Toxics Release Inventory, releases to the air from manufacturing and processing facilities were about 6,7 million pounds (3,055 metric tons) in 2004 (TRI04 2006). Releases to air accounted for about 93% of the total industry-related releases to the environment (TRI04 2006). Benzene is released to water and soil from industrial discharges, landfill leachate, and gasoline leaks from underground storage tanks.

Chemical degradation reactions, primarily reaction with hydroxyl radicals, limit the atmospheric residence time of benzene to only a few days, and possibly to only a few hours. Benzene released to soil or waterways is subject to volatilization, photooxidation, and biodegradation. Biodegradation, principally under aerobic conditions, is an important environmental fate process for water- and soil-associated benzene.

Benzene is ubiquitous in the atmosphere. It has been identified in air samples of both rural and urban environments and in indoor air. Although a large volume of benzene is released to the environment, environmental levels are low because of efficient removal and degradation processes. Benzene partitions mainly into air (99.9%) and inhalation is the dominant pathway of human exposure accounting for >99% of the total daily intake of benzene (Hattemer-Frey et al. 1990; MacLeod and MacKay 1999). The general population is exposed to benzene primarily by tobacco smoke (both active and passive smoking) and by inhaling contaminated air (particularly in areas with heavy motor vehicle traffic and around filling stations). Air around manufacturing plants that produce or use benzene and air around landfills and hazardous waste sites that contain benzene are additional sources of exposure. Exposure to benzene can

Figure 6-1. Frequency of NPL Sites with Benzene Contamination



Derived from HazDat 2006

also result from ingestion of contaminated food or water. Use of contaminated tap water for cooking, showering, etc., can also be a source of inhalation exposure since benzene can volatilize from water. Compared to inhalation, dermal exposure accounts for a minor portion of the total exposure of the general population. The magnitude of exposure is greatest for those individuals occupationally exposed to benzene; however, a far greater number of individuals are exposed as a result of benzene released from smoking tobacco products, from gasoline filling stations, and from auto exhaust. Smoking was found to be the largest anthropogenic source of direct human exposure to benzene (Duarte-Davidson et al. 2001; Hattemer-Frey et al. 1990).

6.2 RELEASES TO THE ENVIRONMENT

The Toxics Release Inventory (TRI) data should be used with caution because only certain types of facilities are required to report (EPA 2005g). This is not an exhaustive list. Manufacturing and processing facilities are required to report information to the TRI only if they employ 10 or more full-time employees; if their facility is included in Standard Industrial Classification (SIC) Codes 10 (except 1011, 1081, and 1094), 12 (except 1241), 20–39, 4911 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4931 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4939 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4953 (limited to facilities regulated under RCRA Subtitle C, 42 U.S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited to facilities primarily engaged in solvents recovery services on a contract or fee basis); and if their facility produces, imports, or processes ≥25,000 pounds of any TRI chemical or otherwise uses >10,000 pounds of a TRI chemical in a calendar year (EPA 2005g).

6.2.1 Air

Estimated releases of 6.7 million pounds (~ 3,055 metric tons) of benzene to the atmosphere from 968 domestic manufacturing and processing facilities in 2004, accounted for about 93% of the estimated total environmental releases from facilities required to report to the TRI (TRI04 2006). These releases are summarized in Table 6-1.

Benzene is released into the atmosphere from both natural and industrial sources. Natural sources include crude oil seeps, forest fires, and plant volatiles (Brief et al. 1980; Graedel 1978). Major anthropogenic sources of benzene include environmental tobacco smoke, automobile exhaust, automobile refueling

Table 6-1. Releases to the Environment from Facilities that Produce, Process, or Use Benzene[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
AK	7	19,568	19	0	375	0	19,962	0	19,962
AL	15	290,660	290	0	280	2	290,955	277	291,232
AR	8	33,137	66	0	0	0	33,203	0	33,203
AZ	13	4,910	0	0	9	0	4,915	4	4,919
CA	65	57,611	129	228	1,292	598	57,970	1,888	59,858
CO	11	19,630	0	0	10	36	19,630	46	19,676
CT	9	2,552	2	0	0	2	2,554	2	2,556
DE	5	3,425	6,006	0	26	0	9,431	26	9,457
FL	26	396,187	41	0	0	157	396,228	157	396,385
GA	15	36,893	128	0	9	0	37,026	4	37,030
GU	3	7,106	1	0	0	401	7,107	401	7,508
HI	9	11,171	30	0	12	0	11,201	12	11,213
IA	14	42,671	1	0	0	0	42,672	0	42,672
ID	2	1,732	No data	0	0	0	1,732	0	1,732
IL	35	171,104	102	0	2,152	104	172,227	1,235	173,462
IN	30	211,476	805	14,001	4,654	29	227,079	3,886	230,965
KS	19	69,823	164	231	259	0	70,226	251	70,477
KY	22	77,345	799	0	766	20	78,904	26	78,930
LA	62	605,128	1,075	122,723	1,688	968	729,109	2,473	731,582
MA	10	5,556	55	0	26	537	5,611	563	6,173
MD	11	12,644	8	0	184	1	12,821	15	12,836
ME	5	3,027	23	0	0	467	3,049	467	3,516
MI	41	345,255	8	0	127	2,295	345,363	2,322	347,686
MN	16	16,614	2,724	0	131	1,561	19,360	1,670	21,030
MO	17	98,498	0	0	0	0	98,498	0	98,498
MP	2	710	0	0	0	0	710	0	710
MS	13	59,076	25	0	14	0	59,102	13	59,115
MT	5	17,242	5	0	15	0	17,248	14	17,262
NC	12	17,290	0	0	5	48	17,295	48	17,343
ND	2	6,400	0	0	1	0	6,400	1	6,401
NE	9	1,333	No data	0	0	6	1,333	6	1,339
NJ	20	51,054	443	0	150	169	51,510	306	51,816
NM	9	38,730	No data	7	2	0	38,739	0	38,739
NV	3	1,005	No data	0	0	0	1,005	0	1,005
NY	44	60,682	56	0	10	1,335	60,738	1,345	62,084
OH	52	183,972	67	5	730	82	184,049	807	184,856
OK	11	114,701	14	0	278	71	114,984	80	115,064

Table 6-1. Releases to the Environment from Facilities that Produce, Process, or Use Benzene[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
OR	6	4,651	8	0	41	0	4,659	41	4,700
PA	39	234,647	419	0	345	11,389	235,066	11,733	246,800
PR	10	49,879	7	0	0	0	49,886	0	49,886
RI	2	1,841	4	0	0	820	1,845	820	2,665
SC	6	64,627	6	0	250	1,023	64,883	1,023	65,906
SD	11	272	1	0	0	0	273	0	273
TN	17	73,926	63	0	280	0	73,989	280	74,269
TX	150	2,681,863	621	298,595	8,326	1,713	2,935,539	55,579	2,991,118
UT	11	31,314	750	0	809	233	32,069	1,038	33,107
VA	17	183,748	787	0	26	0	184,535	26	184,561
VI	3	20,576	0	0	9	0	20,576	9	20,585
WA	18	50,667	14	0	95	48	50,770	54	50,824
WI	14	141,183	0	0	251	63	141,183	314	141,496
WV	5	76,556	284	0	393	0	76,840	393	77,233
WY	7	24,986	0	0	1	0	24,986	1	24,987
Total	968	6,736,655	16,051	435,790	24,033	24,179	7,147,049	89,659	7,236,707

[a]The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list. Data are rounded to nearest whole number.
[b]Data in TRI are maximum amounts released by each facility.
[c]Post office state abbreviations are used.
[d]Number of reporting facilities.
[e]The sum of fugitive and point source releases are included in releases to air by a given facility.
[f]Surface water discharges, waste water treatment-(metals only), and publicly owned treatment works (POTWs) (metal and metal compounds).
[g]Class I wells, Class II-V wells, and underground injection.
[h]Resource Conservation and Recovery Act (RCRA) subtitle C landfills; other on-site landfills, land treatment, surface impoundments, other land disposal, other landfills.
[i]Storage only, solidification/stabilization (metals only), other off-site management, transfers to waste broker for disposal, unknown
[j]The sum of all releases of the chemical to air, land, water, and underground injection wells.
[k]Total amount of chemical transferred off-site, including to POTWs.

RF = reporting facilities; UI = underground injection

Source: TRI04 2006 (Data are from 2004)

operations, and industrial emissions. Using source exposure modeling, it was estimated that benzene emissions were highest from coke oven blast furnaces (Edgerton and Shah 1992). Other sources that contributed to emissions of benzene include automobiles, petrochemical industries, waste water treatment plants, and petroleum industries (Edgerton and Shah 1992).

Industrial and automotive sources of benzene are well monitored. EPA (1989) estimates of nationwide benzene atmospheric emissions, in metric tons/year (kkg/year), from various industrial sources include: (1) coke byproduct recovery plants (17,000 kkg/year), (2) benzene waste operations (5,300 kkg/year), (3) gasoline marketing systems, including bulk gasoline terminals and plants, service stations, and delivery tank trucks (4,800 kkg/year), (4) transfer operations at chemical production facilities, bulk terminals, and coke byproduct recovery plants (4,600 kkg/year), (5) benzene storage vessels (620–1,290 kkg/year), (6) industrial solvent use (450 kkg/year), (7) chemical manufacturing process vents (340 kkg/year), and (8) ethylbenzene/styrene process vents (135 kkg/year). Benzene is present in passenger car tailpipe emissions at compositions ranging from 2.9 to 15% of the total tailpipe hydrocarbon composition (Black et al. 1980). The 2002 benzene industrial emission inventory for California totaled 266 metric tons/year (CARB 2005); these numbers did not include motor vehicle exhaust,which accounted for 71% of emissions in 1984 (Cal EPA 1987). The contribution of mobile source hazardous air pollutant emissions has been compared to that of stationary sources in the Seattle-Tacoma area. Mobile sources were estimated to contribute approximately 83% of the benzene from stationary areas and mobile sources combined, with major stationary point sources excluded (EPA 1994d). Benzene is also released by off-gassing from particle board (Glass et al. 1986), vaporization from oil spills, and emissions from landfills (Bennett 1987; Wood and Porter 1987). While all of these sources release more benzene into the environment, a large percentage of the benzene inhaled by humans comes from cigarette smoke. Exhaled breath of smokers contains benzene (Wallace 1989a, 1989b; Wallace and Pellizzari 1986; Wester et al. 1986).

According to the Texas Natural Resource Conservation Commission (TNRCC), of the 93 districts in Texas reporting benzene emissions from industrial sources, in 1980, 1985, and 1988, 10 districts reported 0 tons/year, 79 districts reported 0.1–100 tons/year, 4 districts reported 100–400 tons/year, 2 districts reported 400–1,000 tons/year, and 1 district had emissions projected at above 1,000 tons/year (Pendleton 1995).

The natural sources most monitored for benzene released to air are fires (Austin et al. 2001; Lowry et al. 1985). A study of nine municipal fires in Canada found a mean concentration of 3.45 ppm of benzene

and had very high relative concentration of other volatile organic compounds (VOCs) monitored (Austin et al. 2001).

There is a potential for atmospheric release of benzene from hazardous waste sites. Benzene has been detected in air in 200 of the 1,684 current and former NPL sites where it has been detected in some medium (HazDat 2006).

6.2.2 Water

Estimated releases of 16,051 pounds (~7 metric tons) of benzene to surface water from 968 domestic manufacturing and processing facilities in 2004, accounted for about 0.2% of the estimated total environmental releases from facilities required to report to the TRI (TRI04 2006). These releases are summarized in Table 6-1.

Benzene is released to water from the discharges of both treated and untreated industrial waste water, gasoline leaks from underground storage tanks, accidental spills during marine transportation of chemical products, and leachate from landfills and other contaminated soils, (CDC 1994; Crawford et al. 1995; EPA 1979; NESCAUM 1989; Staples et al. 1985). A fire in a tire dump site in western Frederick County, Virginia, produced a free-flowing oily tar containing benzene among other chemicals. The seepage from this site contaminated nearby surface water (EPA 1993). Between 1986 and 1991, 3,000 gallons of benzene were accidentally released into Newark Bay and its major tributaries. Another 3,000 gallons were released in 1991 (Crawford et al. 1995).

There is a potential for release of benzene to water from hazardous waste sites. Benzene has been detected in groundwater samples collected at 832 of the 1,684 current and former NPL sites and in surface water samples collected at 208 of the 1,684 sites (HazDat 2006).

6.2.3 Soil

Estimated releases of 24,033 pounds (~11 metric tons) of benzene to soils from 968 domestic manufacturing and processing facilities in 2004, accounted for about 0.3% of the estimated total environmental releases from facilities required to report to the TRI (TRI04 2006). An additional 435,000 pounds (~197 metric tons), constituting about 6% of the total environmental emissions, were released via underground injection (TRI04 2006). These releases are summarized in Table 6-1.

Benzene is released to soils through industrial discharges, land disposal of benzene-containing wastes, and gasoline leaks from underground storage tanks. In northern Virginia, approximately 200,000 gallons of liquid hydrocarbons were released from a fuel-storage terminal into the underlying soil (Mushrush et al. 1994).

There is a potential for release of benzene to soil from hazardous waste sites. Benzene has been detected in soil samples collected at 436 of the 1,684 sites, and in sediment samples collected at 145 of the 1,684 sites where benzene has been detected in some medium (HazDat 2006).

6.3 ENVIRONMENTAL FATE

6.3.1 Transport and Partitioning

The high volatility of benzene is the controlling physical property in the environmental transport and partitioning of this chemical. Benzene is considered to be highly volatile with a vapor pressure of 95.2 mm Hg at 25 °C. Benzene is moderately soluble in water, with a solubility of 1,780 mg/L at 25 °C, and the Henry's law constant for benzene ($5.5x10^{-3}$ atm-m^3/mole at 25 °C) indicates that benzene partitions readily to the atmosphere from surface water (Mackay and Leinonen 1975). Since benzene is soluble in water, some minor removal from the atmosphere via wet deposition may occur. A substantial portion of any benzene in rainwater that is deposited to soil or water will be returned to the atmosphere via volatilization.

Benzene released to soil surfaces partitions to the atmosphere through volatilization, to surface water through runoff, and to groundwater as a result of leaching. The soil organic carbon sorption coefficient (K_{oc}) for benzene has been measured with a range of 60–83 (Karickhoff 1981; Kenaga 1980), indicating that benzene is highly mobile in soil and readily leaches into groundwater. Other parameters that influence leaching potential include the soil type (e.g., sand versus clay), amount of rainfall, depth of the groundwater, and extent of degradation. In a study of the sorptive characteristics of benzene to groundwater aquifer solids, benzene showed a tendency to adsorb to aquifer solids. Greater soil adsorption was observed with increasing organic matter content (Uchrin and Mangels 1987). An investigation of the mechanisms governing the rates of adsorption and desorption of benzene by dry soil grains revealed that periods of hours are required to achieve equilibrium and that adsorption is much faster than desorption (Lin et al. 1994). The rate of volatilization and leaching are the principal factors that determine overall persistence of benzene in sandy soils (Tucker et al. 1986).

Studies suggest that benzene does not bioaccumulate in marine organisms. The bioconcentration/ bioaccumulation potential of benzene in aquatic organisms of the open coastal ocean was investigated by sampling final effluent from the Los Angeles County waste water treatment plant quarterly from November 1980 to August 1981 (Gossett et al. 1983). Benzene has a relatively low octanol/water partition coefficient (log K_{ow}=2.13 or 2.15) (Gossett et al. 1983; HSDB 2007). In the alga, *Chlorella*, a bioaccumulation factor of 30 was determined experimentally (Geyer et al. 1984). An experimental bioconcentration factor (BCF) of 4.27 was measured in goldfish reared in water containing 1 ppm of benzene (Ogata et al. 1984). Based on these measured values, bioconcentration/bioaccumulation of benzene in the aquatic food chains does not appear to be important. These results are consistent with the fact that benzene has a relatively low octanol/water partition coefficient (Gossett et al. 1983; HSDB 2007), suggesting relatively low bioaccumulation. There is no evidence in the literature of biomagnification of benzene in aquatic food chain.

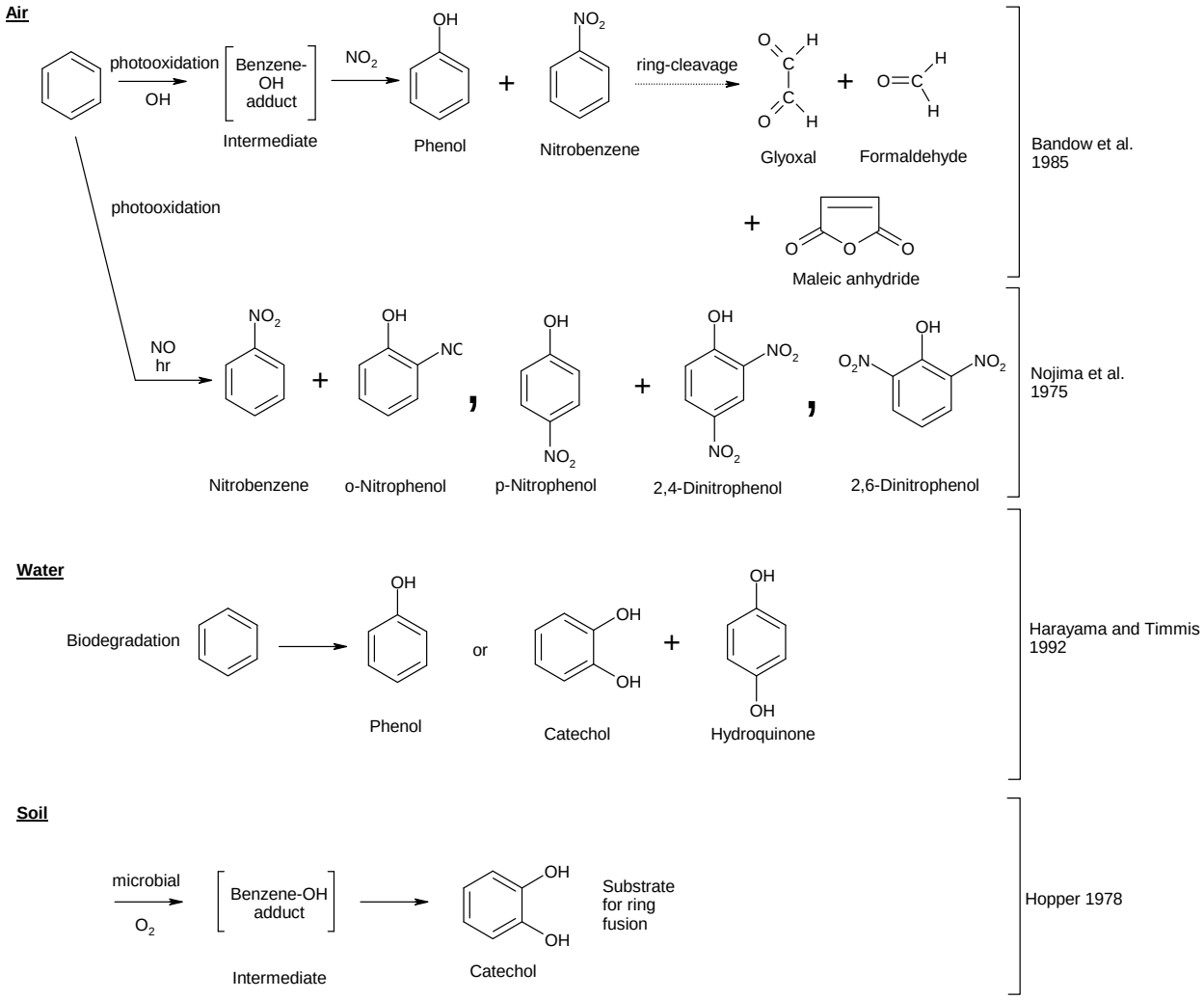
Evidence exists for the uptake of benzene by cress and barley plants from soil (Scheunert et al. 1985; Topp et al. 1989). BCFs for barley plants after 12, 33, 71, and 125 days were 17, 2.3, 2.9, and 4.6, respectively. BCFs for cress plants after 12, 33, and 79 days were 10, 2.3, and 1.9, respectively. The relative decrease in the BCFs with time was attributed to growth dilution (Topp et al. 1989). Since benzene exists primarily in the vapor phase, air-to-leaf transfer is considered to be the major pathway of vegetative contamination (Hattemer-Frey et al. 1990). Based on an equation to estimate vegetative contamination, the total concentration of benzene on exposed food crops consumed by humans and used as forage by animals was estimated to be 587 ng/kg, 81% of which was from air-to-leaf transfer and 19% was from root uptake (Hattemer-Frey et al. 1990).

Benzene also accumulates in leaves and fruits of plants. After 40 days, plants grown in benzene-rich environments showed bioaccumulation in the leaves and fruit that were greater than the air portioning coefficient of benzene in the atmosphere. Blackberries exposed to 0.313 ppm and apples exposed to 2.75 ppm contained about 1,000 and 36 ng/g of benzene, respectively (Collins et al. 2000).

6.3.2 Transformation and Degradation

Benzene undergoes a number of different transformation and degradation reactions in the environment as discussed in the following sections. The resulting environmental transformation products within different media are shown in Figure 6-2.

Figure 6-2. Environmental Transformation Products of Benzene in Various Media



6.3.2.1 Air

Benzene in the atmosphere exists predominantly in the vapor phase (Eisenreich et al. 1981). The most significant degradation process for benzene is its reaction with atmospheric hydroxyl radicals. The rate constant for the vapor phase reaction of benzene with photochemically produced hydroxyl radicals has been determined to be $1.3x10^{-12}$ cm^3/molecule-second, which corresponds to a residence time of 8 days at an atmospheric hydroxyl radical concentration of $1.1x10^6$ molecules/cm^3 (Gaffney and Levine 1979; Lyman 1982). With a hydroxyl radical concentration of $1x10^8$ molecules/cm^3, corresponding to a polluted atmosphere, the estimated residence time is shortened to 2.1 hours (Lyman 1982). Benzene may also react with other oxidants in the atmosphere such as nitrate radicals and ozone; however, the rate of degradation is considered insignificant compared to the rate of reaction with hydroxyl radicals. Residence times of 472 years for rural atmospheres and 152 years for urban atmospheres were calculated for the reaction of benzene with ozone (O_3) using a rate constant for O_3 of $7x10^{-23}$ cm^3/molecule-second (Pate et al. 1976) and atmospheric concentrations for O_3 of $9.6x10^{11}$ molecules/cm^3 (rural) and $3x10^{12}$ molecules/cm^3 (urban) (Lyman 1982).

The reaction of benzene and nitric oxide in a smog chamber was investigated to determine the role of benzene in photochemical smog formation (Levy 1973). The results showed that benzene exhibited low photochemical smog reactivity in the four categories tested: rate of photooxidation of nitric oxide, maximum oxidant produced, eye-irritation response time, and formaldehyde formation. The authors concluded that benzene probably does not play a significant role in photochemical smog formation (Levy 1973). In the presence of active species such as nitrogen oxides and sulfur dioxide, the rate of photodegradation of benzene in the gas phase was greater than that in air alone. Its half-life in the presence of such active species (100 ppm benzene in the presence of 10–110 ppm NO_x or 10–100 ppm SO_2) was 4–6 hours with 50% mineralization to carbon dioxide in approximately 2 days (Korte and Klein 1982). Some of the products of the reaction of benzene with nitrogen monoxide gas (e.g., nitrobenzene, *o*- and *p*-nitrophenol, and 2,4- and 2,6-dinitrophenol) may have potentially adverse effects on human health (Nojima et al. 1975); however, these species also have relatively short atmospheric lifetimes, which should limit the potential exposure to these compounds. Photooxidation of benzene in a nitrogen monoxide/nitrogen dioxide-air system formed formaldehyde, formic acid, maleic anhydride, phenol, nitrobenzene, and glyoxal (ethane-1,2-dione) (Bandow et al. 1985).

Direct photolysis of benzene in the atmosphere is not likely because the upper atmosphere effectively filters out wavelengths of light <290 nm, and benzene does not absorb wavelengths of light >260 nm (Bryce-Smith and Gilbert 1976).

6.3.2.2 Water

Benzene is subject to indirect photolysis in sunlit surface water, but does not undergo direct photolysis. For direct photolysis to occur, a substance must absorb photons of light >290 nm. During indirect photolysis, light energy is absorbed by other constituents (photosensitizers) of the media (water, soil) and the excited species can then transfer energy to benzene (indirectly promoting it to an excited electronic state), or lead to the formation of reactive species, such as singlet oxygen or hydroxy radicals, which react with benzene. Humic and fulvic acids are well-known photosensitizing agents and are practically ubiquitous in natural waters. A half-life of 16.9 days was reported for photolysis of benzene dissolved in oxygen-saturated deionized water and exposed to sunlight (Hustert et al. 1981).

Benzene is readily degraded in water under aerobic conditions. Results of a biochemical oxygen demand (BOD) test determined that benzene was completely biodegradable after the second week of static incubation at 25 °C at benzene concentrations of 5 and 10 mg/L using domestic waste water as the microbial inoculum (Tabak et al. 1981). A study of the degradation of benzene by the microbial population of industrial waste water at 23 °C using a shaker flask system showed that after 6 hours, only 8% (4 mg/L) of the initial 50 mg/L dose of benzene remained (Davis et al. 1981). Water from a petroleum production site was successfully biotreated for complete removal of benzene using a flocculated culture of *Thiobacillus denitrificans* strain F and mixed heterotrophs (Rajganesh et al. 1995).

Microbial degradation of benzene in aquatic environments is influenced by many factors including microbial population, dissolved oxygen, nutrients, other sources of carbon, inhibitors, temperature, pH, and initial concentration of benzene. Vaishnav and Babeu (1987) reported biodegradation half-lives for benzene in surface water (river water) and groundwater of 16 and 28 days, respectively. Benzene was found to be resistant to biodegradation in surface water taken from a harbor and supplemented with either nutrients (nitrogen and phosphorus) or acclimated microbes, however, biodegradation did occur, with a half-life of 8 days, in surface water enriched with both nutrients and microbes (Vaishnav and Babeu 1987). At very high levels, as may be the case of a petroleum spill, benzene (and other compounds contained in petroleum) is toxic to microorganisms and the rate of degradation is slow compared to low initial starting concentrations. In another study, Davis et al. (1994) observed rapid aerobic biodegradation

of benzene in aquifer groundwater samples and measured times for 50% disappearance ranging from 4 days for an initial benzene concentration of 1 mg/kg to 14 days for an initial benzene concentration of 10 mg/kg. Under acidic conditions (pH 5.3, 20 °C), benzene was completely microbially degraded in 16 days in groundwater taken from a shallow well (Delfino and Miles 1985).

The aerobic biodegradation of benzene is also influenced by the presence of other aromatic hydrocarbons. A bacterial culture grown with aromatic hydrocarbons plus nitrogen-, sulfur-, and oxygen-containing aromatic compounds was much less efficient in degrading benzene than the culture grown with aromatic hydrocarbons alone. Pyrrole strongly inhibited benzene degradation. Benzene degradation was high when toluene and xylene were present (Arvin et al. 1989).

Laboratory studies on microbial degradation of benzene with mixed cultures of microorganisms in gasoline-contaminated groundwater revealed that both oxygen and nitrogen concentrations are major controlling factors in the biodegradation of benzene. Nitrogen enhanced the biodegradation rate of benzene 4.5-fold, over innoculum-enriched water alone. More than 95% of the benzene in groundwater was removed through microbial action within 73.5 hours (Karlson and Frankenberger 1989).

Benzene biodegradation under anaerobic conditions does not readily occur. When dissolved oxygen is depleted, an alternative electron acceptor such as nitrate, carbonate, or iron(III) must be available, and microbes capable of using the alternative electron acceptor to degrade the benzene must be present (McAllister and Chiang 1994). Using aquifer material obtained from a landfill from Norman, Oklahoma, no significant benzene biodegradation was reported during the first 20 weeks of incubation under anaerobic conditions at 17 °C; however, after 40 weeks of incubation, benzene concentrations were reduced by 72 and 99% of the benzene was degraded after 120 weeks (Wilson et al. 1986). No degradation of benzene was observed in 96 days under anaerobic conditions (20 °C) using raw water intake from a water treatment plant (Delfino and Miles 1985).

Use of water as an oxygen source in the anaerobic degradation of benzene has been demonstrated. Experiments indicated that incorporation of ^{18}O from ^{18}O-labeled water is the initial step in the anaerobic oxidation of benzene by acclimated methanogenic cultures. Phenol was the first major product (Vogel and Grbić-Galić 1986).

6.3.2.3 Sediment and Soil

Benzene is biodegraded in soil under aerobic conditions. Microbial metabolism of benzene proceeds through the formation of *cis*-dihydrodiols and, with further metabolism, to catechols, which are the substrates for ring fission (Gibson 1980; Hopper 1978). *Pseudomonas putida* oxidized benzene through *cis*-1,2-dihydroxy-1,2-dihydrobenzene (Gibson 1977; Hopper 1978). After acclimation, *Norcardia* species and *Pseudomonas* species, effectively degraded benzene to carbon dioxide within 7 days (45–90%). A strain of *Rhodococcus* isolated from contaminated river sediment mineralized 71% of benzene at an initial concentration of 0.7 mg/L in 14 days (Malachowsky et al. 1994). The soil bacterium *Nitrosomonas europaea* catabolized benzene to phenol and hydroquinone (Keener and Arp 1994). Another new mixotrophic bacteria, a strain of *Pseudomonas sp.* isolated from contaminated soil, grew under both anaerobic and aerobic conditions and used benzene for its growth (Morikawa and Imanaka 1993). The biodegradation of 2 mg of radiolabeled benzene in 100 g of soil with a mixed microbial population transformed 47% of the added radioactivity to carbon dioxide after 10 weeks (Haider et al. 1981). The authors concluded that specific organisms that mineralize benzene were present in the soil in only small numbers.

ULTRA, a fate and transport model used to predict the environmental fate of benzene following leakage of gasoline from an underground storage tank into shallow sandy soil, indicated that only about 1% of the benzene in the gasoline would be degraded over a 17-month period, and 3% would remain in the soil (Tucker et al. 1986). According to this model, most of the benzene that would be leaked in soil would either volatilize (67%) or move into groundwater (29%).

Salanitro (1993) summarized the aerobic degradation rates for BTEX in laboratory subsoil-groundwater slurries and aquifers. The data indicate that decay rates for benzene are highest (19–52% per day) for benzene concentrations <1 ppm when initial dissolved oxygen levels are about 8 ppm. Rates are significantly reduced (0–1.1% per day) when benzene levels are 1–2 ppm, and no degradation was observed when benzene levels were >2 ppm. This is particularly relevant in the case of petroleum spills as high concentrations of petroleum compounds are toxic to organisms and decrease the rate of biodegradation.

Benzene has been shown to be anaerobically transformed by mixed methanogenic cultures derived from ferulic acid-degrading sewage sludge enrichments. In most of the experiments, benzene was the only semicontinuously supplied energy sources in the defined mineral medium (Grbić-Galić and Vogel 1987).

After an initial acclimation time of 11 days, at least 50% of the substrate was converted to CO_2 and methane. The intermediates were consistent with benzene degradation via initial oxidation by ring hydroxylation.

It has been demonstrated that when mixtures of benzene, toluene, xylenes, and ethylbenzene are present in an anaerobic environment, there is a sequential utilization of the substrate hydrocarbons, with toluene usually being the first to be degraded, followed by the isomers of xylene in varying order. Benzene and ethylbenzene tend to be degraded last, if they are degraded at all (Edwards and Grbić-Galić 1992). Similar studies using only benzene at initial concentrations ranging from 40 to 200 μM showed degradation rates ranging from 0.36 to 3.7 μM/day depending upon substrate concentration and the presence of other carbon sources (Edwards and Grbić-Galić 1992).

6.3.2.4 Other Media

Twenty-day-old spinach leaves placed in a hermetic chamber containing vapors of ^{14}C-labeled benzene were shown to assimilate benzene, which was subsequently metabolized to various nonvolatile organic acids (Ugrekhelidze et al. 1997).

6.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

Reliable evaluation of the potential for human exposure to benzene depends in part on the reliability of supporting analytical data from environmental samples and biological specimens. Concentrations of benzene in unpolluted atmospheres and in pristine surface waters are often so low as to be near the limits of current analytical methods. In reviewing data on benzene levels monitored or estimated in the environment, it should also be noted that the amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable. The analytical methods available for monitoring benzene in a variety of environmental media are detailed in Chapter 7.

6.4.1 Air

Benzene is ubiquitous in the atmosphere. It has been identified in outdoor air samples of both rural and urban environments and in indoor air. Table 6-2 lists benzene levels in outdoor air from various cities in the United States.

Table 6-2. Benzene Levels in Air Samples

Location	Concentration (ppb)	Comments	References
Outdoor air			
San Francisco, California	0.8–5.2 (range); 2.6±1.3[a]	Data from six different urban locations in 1984; n=25	Wester et al. 1986
Stinson Beach, California	0.38±0.39[a]	Data from remote coastal area in 1984; n=21	Wester et al. 1986
Houston, Texas	1.2–18.7 (range); 37.5[b]	EPA survey, urban area; n=14; summer 1986	EPA 1987a[c]
Houston, Texas	0.55–6.3; 1.73[b]	Semirural area; n=22; 1990–1991	Kelly et al. 1993
St. Louis, Missouri	0.63–12.1 (range); 1.85[b]	EPA survey, urban area; n=18; summer 1985	EPA 1987a[c]
Denver, Colorado	3.0–6.6 (range); 4.1[b]	EPA survey, urban area; n=13; summer 1986	EPA 1987a[c]
Philadelphia, Pennsylvania	0.32–3.0 (range); 1[b]	EPA survey, urban area; n=14; summer 1985	EPA 1987a[c]
New York (Manhattan), New York	0.88–5.3 (range); 1.75[b]	EPA survey, urban area; n=12; summer 1986	EPA 1987a[c]
Chicago, Illinois	0.63–5.05 (range); 3.45[b]	EPA survey, urban area; n=14; summer 1986	EPA 1987a[c]
Boston, Massachusetts	0.69–3.1; 1.06[b]	Urban area; n=22; 1990–1991	Kelly et al. 1993
New York (Staten Island), New York	0.1–34 (range); 4.4±6.6[a]	Urban area; spring 1984	Singh et al. 1985
73 km northwest of Denver, Colorado	0.02–0.85 (range)	Rural area; May 1981–December 1982	Roberts et al. 1985
Elizabeth and Bayonne, New Jersey	Night 2.7[d], 28.5 (max); day, 3.0[d], 13.8 (max)	Night, n=81–86; day, n=86–90; fall 1981	Wallace et al. 1985
Personal air			
Elizabeth and Bayonne, New Jersey	Night 9.7[d], 159.6 (max); day, 8.5[d], 84.5 (max)	Night, n=346–348; day, n=339–341; fall 1981	Wallace et al. 1985
Elizabeth and Bayonne, New Jersey	Winter 5.2, summer 2.7, fall 4.8		Rappaport and Kupper 2004
Los Angles, California	Winter 4.5, spring 2.2, fall 2.8		Rappaport and Kupper 2004

[a]Average±standard deviation
[b]Median
[c]Concentrations reported as ppb carbon; values presented in table represent conversion equivalents as ppb benzene.
[d]Weighted average

EPA = Environmental Protection Agency; max = maximum; N = number of samples

Volatile organic compounds, including benzene, were measured at 11 monitoring sites in Anchorage, Alaska, in a year-long study ending in April 1994 (Taylor and Morris 1995). Average annual benzene concentrations ranged from a minimum of 1.15 parts per billion by volume (ppbv) in a low density residential area to 5.44 ppbv near a major midtown intersection. In a neighborhood where residents were complaining of petroleum odors, the highest benzene concentrations (annual average 3.74 ppbv) were measured on a cliff over the petroleum tank farm, within 50 meters of a petroleum storage tank.

Ambient air samples from 44 sites in 39 U.S. urban areas were collected from 6 to 9 a.m. during June through September of 1984, 1985, and 1986. Benzene was present in every sample. The median benzene site concentrations ranged from 0.8 to 6 ppb, with the overall median being 2.1 ppb (estimated detection limit ~0.04 ppb carbon; conversion equivalent ≈0.007 ppb benzene). The data indicated that mobile sources were the major source of benzene in the vast majority of samples (EPA 1987a). Benzene concentrations were in the range of >1–<5 ppbv for all of the 13 sites tested in a 1996 study. The sites included: Baton Rouge, Louisiana; Brownsville, Texas; Brattleboro, Vermont; Burlington, Vermont; Camden, New Jersey; El Paso, Texas; Garyville, Louisiana; Galveston, Texas; Hanville, Louisiana; Port Neches, Texas; Rutland, Vermont; Underhill, Vermont; and Winooski, Vermont (Mohamed et al. 2002).

The following daily median benzene air concentrations were reported in the Volatile Organic Compound National Ambient Database (1975–1985): remote (0.16 ppb), rural (0.47 ppb), suburban (1.8 ppb), urban (1.8 ppb), indoor air (1.8 ppb), and workplace air (2.1 ppb). The outdoor air data represent 300 cities in 42 states while the indoor air data represent 30 cities in 16 states (Shah and Singh 1988). In a NHEXAS study of six states in the Great Lakes region, benzene was found in 99.7% of the 386 personal air samples taken with an average concentration of 2.35 ppb. Benzene was also found in 100% of both indoor and outdoor samples with concentrations averaging 2.25 and 1.13 ppb, respectively (Clayton et al. 1999).

An EPA study of the concentration of various pollutants in 1990 showed an average concentration of benzene of 0.948 ppb. The source of benzene included manufacturing facilities and manufacturers that emit benzene to the environment (17%), mobile (60%), and background (23%) sources (Morello-Frosch et al. 2000).

In California, motor vehicle exhaust accounted for over 70% of the nonsmoking population's exposure to ambient benzene (Cal EPA 1987). The 1984 population-weighted average benzene concentration in California was estimated to be 3.3 ppb (Cal EPA 1987). Benzene emissions in a Los Angeles roadway tunnel were measured at a concentration of 382 mg/L (118,420 ppm) (Fraser et al. 1998).

Industrial sites that produce benzene are monitored often. Analysis of ambient air samples collected in industrial areas showed benzene levels ranging from 0.13 to 5 ppb in Iberville Parish, Louisiana, an area that included many organic chemical and petroleum producer, user, and storage facilities located along the Mississippi River (Pellizzari 1982). Indoor and outdoor air measurements were made in August 1987 in the Kanawha Valley region of West Virginia, which is the center of a heavily industrialized area known for its chemical manufacturing. The mean, maximum, and median indoor concentrations of benzene were 2.1, 14.9, and 0.64 ppb, respectively. The median outdoor ambient air concentration of benzene was 0.78 ppb (Cohen et al. 1989).

An analysis of gas from 20 Class II (municipal) landfills revealed a maximum concentration of 32 ppm for benzene (Wood and Porter 1987). Benzene was measured in the vicinity of the BKK landfill, a hazardous waste landfill in California, at a maximum concentration of 1.2 ppb (Bennett 1987). Maximum estimated levels of benzene in air near uncontrolled (Superfund) hazardous waste sites were 59.5 ppb at the Kin-Buc Landfill (Edison, New Jersey) and 162.8 ppb in Love Canal basements (Niagara Falls, New York) (Bennett 1987; Pellizzari 1982).

According to the National Cancer Institute, the average concentration of benzene in 1998 was 0.58 ppb in metropolitan areas (NCI 2003). A study of New York City air found mean values of 0.80, 1.87, and 1.47 ppb for outdoor (home), indoor (home), and personal air, respectively (Kinney et al. 2002). According to the New York Department of Health, the air from about 50% of oil fuel heated homes between the years 1997 and 2003 contained benzene concentrations ≥0.69 ppb inside the home, and 0.47 ppb in the area outside their homes. New York City was not included in this study (NYSDOH 2005). Concentrations of VOCs were measured in 12 northern California office buildings with three different types of ventilation. Benzene concentrations ranged from <0.1 to 2.7 ppb, with a geometric mean of 0.98 ppb (Daisey et al. 1994).

Population-weighted personal exposures to benzene exceeded the outdoor air concentrations in data from EPA's Total Exposure Assessment Methodology (TEAM) study. The overall mean personal exposure was about 4.7 ppb, compared to an overall mean outdoor concentration of only 1.9 ppb (Wallace 1989a). The study also reported that the median level of benzene in 185 homes without smokers was 2.2 ppb, and the median level of benzene in 343 homes with one or more smokers was 3.3 ppb (Wallace 1989a). This finding points to the possible significance of passive smoking as a source of benzene exposure. Indoor air samples taken from a smoke-filled bar contained 8.08–11.3 ppb of benzene (Brunnemann et al. 1989). A

study conducted by R.J. Reynolds Tobacco Company in smoking and nonsmoking homes revealed that benzene levels were elevated in smoking homes. In 24 nonsmoking homes, the mean benzene concentration was 1.21 ppb with a maximum of 5.93 ppb. In 25 smoking homes, the mean benzene concentration was 1.73 ppb, with a maximum of 8.43 ppb. However, benzene was not significantly correlated or associated with 3-ethenylpyridine, a proposed vapor phase environmental tobacco smoke marker (Heavner et al. 1995).

A British study measured benzene concentrations from both rural and urban areas in 1995. The highest concentrations of benzene were in Southampton urban center, London roadside, and Liverpool, which contained measured benzene levels of 2.53, 1.69,, and 1.60 ppb, respectively. The lowest measured benzene concentrations were in rural Hartwell and urban Edinborough, which contained 0.4 and 0.69 ppb of benzene, respectively (Duarte-Davidson 2001).

6.4.2 Water

Data from EPA's STOrage and RETrieval (STORET) database (2003–2005) showed that benzene was positively detected in 38% of the surface water samples collected at 571 observation stations ranging from concentrations too low to quantify to 100 μg/L (100 ppb) in one Utah test site. The sampling sites in the STORET database include both ambient and pipe sites. Ambient sites include streams, lakes, ponds, wells, reservoirs, canals, estuaries, and oceans and are intended to be indicative of general U.S. waterway conditions. Pipe sites refer to municipal or industrial influents or effluents (EPA 2005f).

Benzene was found in 904 of the 8,200 samples tested by the U.S. Geological Survey across the United States and Canada (USGS 2005). The EPA found benzene in 13,919 and 1,119 groundwater and surface water samples, respectively (EPA 2001).

Benzene is found in both polluted and unpolluted waters and rainwater. Measured benzene levels in open ocean from the Gulf of Mexico in 1977 in relatively unpolluted and polluted waters were 5–15 ng/kg (5–15 parts per trillion [ppt]), and 5–40 ng/kg (5–40 ppt), respectively (Sauer 1981). Benzene levels measured in coastal surface waters of the Gulf of Mexico were 6 ng/kg (6 ppt) in relatively unpolluted waters and 50–175 ng/kg (50–175 ppt) in polluted coastal waters (Sauer 1981). Benzene has been detected in rainwater in the United Kingdom at a concentration of 87.2 ppb (Colenutt and Thorburn 1980).

Benzene levels in water in the vicinity of five industrial facilities using or producing benzene ranged from <1 ppb to a high of 179 ppb found in plant effluent. In general, benzene in plant effluents quickly dispersed in rivers or streams to levels of 1–2 ppb or less (EPA 1979). The maximum benzene levels observed in monitoring wells in plumes from fuel spills at gasoline service stations ranged from 1,200 to 19,000 ppb (Salanitro 1993). A monitoring well in the vicinity of a bulk storage facility had a maximum benzene level of 45,000 ppb (Salanitro 1993). In northern Virginia, approximately 200,000 gallons of liquid hydrocarbons were released from a fuel-storage terminal into the underlying soil. A dichloromethane extract of groundwater from a monitoring well in the same area gave a benzene concentration of 52.1 ppm (Mushrush et al. 1994). Benzene has been detected at concentrations ranging from 16 to 110 ppb in landfill leachate from a landfill that accepted both municipal and industrial wastes (Cline and Viste 1985).

Composite data from the Comprehensive Emergency Response, Compensation, and Liability Act (CERCLA) monitoring program indicate that benzene was detected at a frequency of 11.2% in groundwater in the vicinity of 178 inactive hazardous waste disposal sites (Plumb 1987). Data from a 1980 national survey by the Council on Environmental Quality on groundwater and surface water contamination showed benzene concentrations in contaminated drinking water wells in New York, New Jersey, and Connecticut ranged from 30 to 330 ppb, with the highest concentration in drinking water from surface water sources reported to be 4.4 ppb (Burmaster 1982). Benzene has also been identified but not quantified as one of the major organic constituents in commercially bottled artisan water in the United States (Dowty et al. 1975). At detection limits ranging from 0.2 to 1.0 ppb, benzene was detected at a concentration of 2 ppb in only one sample of 182 samples of bottled drinking water (Page et al. 1993). In six states of the Great Lakes region, unspecified amounts of benzene were found in 5.7% of the 247 drinking water sites tested (Clayton et al. 1999).

Because the New Jersey Department of Environmental Protection identified serious water issues in a 930 km^2 area within metropolitan Philadelphia, tests have been done to find other water sources such as the Kirkwood-Cohansey aquifer system. A study of 57 water samples taken from the shallow ground water supply at Kirkwood Cohansey aquifer in New Jersey showed that the majority of samples (50) contained between 0.2 and 1 ppbv of benzene, three samples contained <0.1 ppbv, and four samples contained >1 ppbv (Baehr et al. 1999).

6.4.3 Sediment and Soil

A Canadian study estimated that benzene is absorbed by the soil at a rate of 100 tons/year (MacLeod and MacKay 1999). Data from EPA's STORET database (2003–2005) showed that benzene had been positively detected in sediment samples taken at 9% of 355 observation stations with a median level of <5 ppb (EPA 2005f). The concentration of benzene in soil near factories where benzene was produced or used ranged from 2 to 191 ppb (IARC 1982).

Benzene levels ranging from <2 to 191 ppb were recorded in the vicinity of five industrial facilities using or producing benzene (EPA 1979). Sediment levels ranging from 8 to 21 ppb were detected in Lake Pontchartrain in Louisiana (Ferrario et al. 1985). In northern Virginia, approximately 200,000 gallons of liquid hydrocarbons were released from a fuel-storage terminal into the underlying soil. Soil about 1,000 feet from that storage terminal contained benzene gas at a concentration of 1,500 ppm at a depth of 10 feet (Mushrush et al. 1994).

6.4.4 Other Environmental Media

Benzene has been detected in a variety of food. Benzene has been reported to occur in fruits, fish, vegetables, nuts, dairy products, beverages, and eggs (EPA 1982a). Although benzene has been detected in dairy products, there is no evidence of the presence of benzene in either cow's milk or human breast milk (Hattemer-Frey et al. 1990). Eggs had the highest concentrations (2,100 ppb [uncooked] and 500–1,900 ppb [hard-boiled]), followed by haddock (100–200 ppb), Jamaican rum (120 ppb), irradiated beef (19 ppb), heat-treated canned beef (2 ppb), and butter (0.5 ppb). Lamb, mutton, veal, and chicken all had <10 ppb benzene (when the meats were cooked) (EPA 1980b, 1982a). A survey of more than 50 foods collected and analyzed from 1991 to early 1992 (McNeal et al. 1993) revealed that foods (including eggs) without added benzoates contained benzene at levels ≤2 ng/g. The level of benzene in foods containing added benzoates in addition to ascorbates (e.g., imitation strawberry preserves, taco sauce, and duck sauce) ranged from <1 to 38 ng/g. In many foods, the presence of benzene is likely to be due to uptake from the air (Grob et al. 1990). This conclusion was supported by the fact that the uptake decreased with a decrease in exposed surface area of foods and contact time with air (Grob et al. 1990).

The U.S. Food and Drug Administration sponsored a 5-year study to determine the amount of volatile organics in food from 1996 to 2000. The results are shown in Table 6-3 (Fleming-Jones and Smith 2003). Benzene was found in cheddar cheese, cream cheese, margarine, butter, sour cream, ground beef, bologna, hamburger, cheeseburger, pork, beef frankfurters, tuna canned in oil, chicken nuggets, chocolate

Table 6-3. Benzene in Food[a]

Food	Number of cases found (n=14)	Concentration minimum-maximum in ppb
Cheddar cheese	2	20–47
Mixed nuts	3	1–6
Ground beef	12	9–190
Pork bacon	6	2–17
Banana, raw	13	11–132
Frankfurters, beef	4	2–11
Cream cheese	3	1–17
Chocolate cake icing	2	2–23
Tuna canned in oil	7	4–13
Fruit flavored cereal	5	2–21
Eggs scrambled	4	2–40
Peanut butter	5	2–25
Avocado, raw	10	3–30
Popcorn, popped in oil	3	4–22
Blueberry muffin	3	3–8
Strawberries, raw	1	1
Cola carbonated	3	1–138
Orange, raw	2	11–15
Coleslaw with dressing	14	11–102
Sweet roll danish	1	3–3
Potato chips	2	2–7
Fruit flavored sherbet	3	3–61
Quarter pound hamburger cooked	11	4–47
Margarine	1	7
Sandwich cookies	3	1–39
Butter	6	4-22
Chocolate chip cookies	2	1–8
Sour cream	2	3–15
Apple pie fresh/frozen	4	2–11
Chicken nuggets fast food	4	2–5
Graham crackers	2	1–9
French fries fast food	3	2–56
Cheeseburger quarter pound	8	5–44
Cheese pizza	2	1–2
Bologna	4	2–44
Cheese and pepperoni pizza	4	8–30
Olive/safflower oil	6	1–46
Sugar cookies	3	8–30
Cake doughnuts with icing	2	3
Popscicle	4	1–10

[a]Derived from Fleming-Jones and Smith 2003

cake icing, sandwich cookie, chocolate chip cookies, graham crackers, sugar cookies, cake doughnuts with icing, french fries, apple pie, cola carbonated beverages, sweet roll Danish, potato chips, cheese pizza, cheese and pepperoni pizza, mixed nuts, fruit-flavored cereal, fruit flavored sorbet, popsicles, olive/safflower oil, scrambled eggs, peanut butter, popcorn popped in oil, blueberry muffins, coleslaw with dressing, raw banana, avocado, oranges, and strawberries. American cheese was the only food tested that did not contain benzene. Foods with the greatest maximum concentration of benzene included ground beef (maximum 190 ppb), raw bananas (maximum 132 ppb), carbonated cola (maximum 138 ppb), and coleslaw with dressing (maximum 102 ppb).

As part of a program to identify possible exposures that may be important in the high incidence of lung cancer among women in Shanghai, China, Pellizzari et al. (1995) qualitatively identified the volatile components emitted during heating of cooking oils to 265 °C. This study found that, on a relative basis, the intensity of the benzene peak in the total ion current chromatogram of vapors from Chinese rapeseed oil (commonly used in wok cooking) was 14-, 6.6-, and 1.7-fold greater than in vapors from peanut, soybean, and other canola (rapeseed) oils, respectively.

Cigarette smoke remains an important source of human exposure to benzene. The amount of benzene measured in mainstream smoke ranged from 5.9 to 73 μg/cigarette (Brunnemann et al. 1990). Larger amounts of benzene were found in sidestream smoke, ranging from 345 to 653 μg/cigarette (Brunnemann et al. 1990). Benzene has been found in vapor from cigarette smoke in concentrations ranging from 3.2 to 61.2 μg/cigarette depending on the brand of cigarette. The amount of tar in the cigarette was not directly related to the benzene concentration (Darrall et al. 1998).

6.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

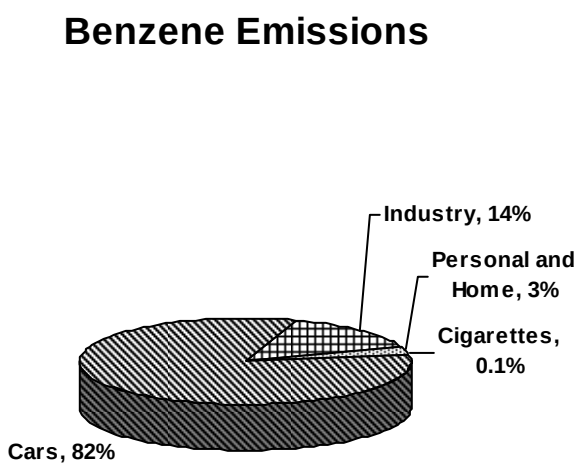
Exposure to low levels of environmental benzene is unavoidable due to the ubiquitous presence of benzene in the environment from a variety of anthropogenic sources. Benzene can be detected in blood samples. In a study designed to establish reference blood concentrations of benzene and other selected volatile organic compounds in the general population of the United States, Ashley et al. (1994) measured blood benzene levels in 883 people (not occupationally exposed) in the United States who had participated in the Third National Health and Nutrition Examination Survey (NHANES III). Within this group of nonoccupationally exposed subjects, the mean and median blood benzene levels were 0.13 and 0.061 ppb, respectively. Seven nonsmoking subjects from this study group were assessed for blood benzene levels just prior to entering examination vans with limited potential for benzene exposure and at

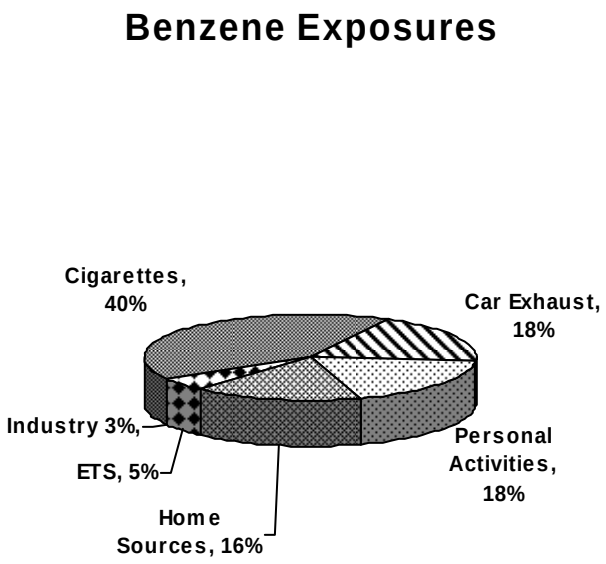
the end of a 3-hour period in this environment. Mean blood benzene levels prior to van entry and after 3 hours in the van measured 0.046 ppb (range from not detected to 0.061 ppb) and 0.033 ppb (range from not detected to 0.055 ppb), respectively. Lemire et al. (2004) reported a median blood benzene level of 0.047 ppb in a subgroup of 546 nonsmoking and nonoccupationally exposed subjects from the NHANES III study group. Elevated blood benzene levels may be expected among subjects with potential for elevated exposure to benzene, such as smokers, commuters, gas station attendants, and people who use products that emit benzene.

The Total Exposure Assessment Monitoring (TEAM) studies, carried out by the EPA between 1980 and 1990, suggested that for many chemicals, including benzene, the most important sources of pollution are small and close to the person, and that exposures are not clearly correlated with emissions. For example, the TEAM study findings indicated that nearly 85% of atmospheric benzene in outdoor air is produced by cars burning petroleum products and the remaining 15% is produced by industry. Despite the fact that petroleum products contribute to the majority of benzene in the atmosphere, half of the total national personal exposure to benzene comes from cigarette smoke (Wallace 1995). In fact, breath measurements of benzene provided by the TEAM study between 1979 and 1988 identified smoking as the single most important source of benzene exposure for about 40 million U.S. smokers (Wallace 1989b). Even passive exposure to cigarette smoke is responsible for more benzene exposure (about 5% of the total) than the emissions from the entire industrial capacity of the United States (about 3% of the total) (Wallace 1995). A breakdown of the emissions and exposure sources for benzene that was derived from the Los Angeles TEAM study data (Wallace et al. 1991) is given in Figure 6-3. The reason that a relatively small source of emissions can have such a large effect on exposure is the efficiency of delivery. Wallace (1995) reports that one cigarette delivers an average of 55 μg of benzene with nearly 100% efficiency to the smoker. Benzene from industrial sources is dissipated into the atmosphere.

Smokers (n=200) in the TEAM study had a mean breath concentration of 15 $\mu g/m^3$ (4.7 ppb), almost 10 times the level of 1.5–2 $\mu g/m^3$ (0.47–0.63 ppb) observed in more than 300 nonsmokers (Wallace 1989b). Smokers also had about 6–10 times as much benzene in their blood as non-smokers (Wallace 1995). In another study, benzene concentrations were compared in the breath of smokers and nonsmokers and in ambient air in both an urban area of San Francisco and in a more remote area of Stinson Beach, California (Wester et al. 1986). In the urban area, benzene in smokers' breath (6.8±3.0 ppb) was greater than in nonsmokers' breath (2.5±0.8 ppb) and smokers' ambient air (3.3±0.8 ppb). In the remote area, the same pattern was observed. This suggests that smoking represented an additional source of benzene above that of outdoor ambient air (Wester et al. 1986). In 10 of 11 homes inhabited by tobacco smokers,

Figure 6-3. Benzene Emissions and Exposures





Note: A comparison of benzene emission sources (home sources include paints and petroleum products; personal activities include driving and use of consumer products that contain benzene). Data taken from Wallace et al. (1991).

mean indoor and personal benzene concentrations were 2–5 times higher than outdoor levels (Thomas et al. 1993).

Assuming that the average sales-weighted tar and nicotine cigarette yields 57 μg benzene in mainstream smoke, Wallace (1989a) estimated that the average smoker (32 cigarettes per day) takes in about 1.8 mg benzene per day from smoking. This is nearly 10 times the average daily intake of nonsmokers (Wallace 1989a). On the assumption that intake of benzene from each cigarette is 30 μg, Fishbein (1992) has calculated that a smoker who consumes two packs of cigarettes per day will have an additional daily intake of 1,200 μg.

A British study of rural and urban environments suggests that benzene exposure is greatly affected by proximity to smokers (Duarte-Davidson et al. 2001). Air concentrations of benzene at an urban center in South Hampton averaged about 2.5 ppb, while in a rural location in Hartwell, the average amount of benzene in the air was 0.41 ppb. Air at a smoky pub was found to contain 22 ppb of benzene. Comparing the daily doses of rural nonsmokers, urban nonsmokers, urban passive smokers, and urban smokers, very little difference between the rural nonsmokers' 24 ppb daily dose and the urban nonsmokers' 30 ppb daily dose was found. Passive urban smokers, on average, have a daily benzene exposure dose of 38 ppb of benzene while smokers have a daily exposure dose of benzene of 163 ppb. On average, nonsmokers in urban and rural environments have estimated benzene intakes of 1.15 and 1.5 μg/kg body weight/day.

Women tend to intake more of benzene per kg body weight than men. Passive smokers' estimated daily intake averages are 2.10 and 1.74 μg/kg body weight/day for women and men, respectively. Urban women and men smokers' estimated intakes are estimated at 9.00 and 7.46 μg/kg body weight/day, respectively; this is equivalent to an atmospheric concentration of 8.2 ppb (Duarte-Davidson et al. 2001).

In 1990, a study in Germany analyzed factors that predicted people's exposures to VOCs and found that while smoking was the most significant determinant of benzene exposure, automobile-related activities, such as refueling and driving, were also significant (Hoffmann et al. 2000). Virtually all (99.9%) of the benzene released into the environment finally distributes itself into the air. The general population may be exposed to benzene through inhalation of contaminated air, particularly in areas of heavy motor vehicle traffic and around gas stations. Compared to inhalation, dermal exposure probably constitutes a minor portion of benzene exposure for the general population. Personal sources account for 18% of the total exposure of the general population to benzene. The main personal sources (other than smoking

cigarettes) are driving or riding in automobiles and using products that emit benzene (paints, adhesives, marking pens, rubber products, and tapes) (Wallace 1989a).

Benzene constitutes 1–2% of most blends of gasoline and is released as a vapor from vehicular emissions. Since benzene is a constituent of auto exhaust and fuel evaporation, people who spend more time in cars or in areas of heavy traffic have increased personal exposure to benzene. Assuming an average benzene concentration of 40 μg/m^3 (12.5 ppb) for a moving automobile and an exposure duration of 1 hour/day, the calculated intake for driving or riding in an automobile is 40 μg/day (Wallace 1989a). In an investigation of exposure to methyl *tertiary*-butyl ether (MTBE) in oxygenated gasoline in Stamford, Connecticut, venous blood samples were collected from 14 commuters and from 30 other persons who worked in the vicinity of traffic or automobiles. In addition to MTBE, the samples were analyzed for five chemicals, including benzene. Levels of benzene in the blood of 11 nonsmoking men and women commuters ranged from 0.10 to 0.20 μg/L (median=0.12 μg/L). Blood benzene levels of 0.29, 0.14, and 0.58 μg/L were measured in one female and two male smoking commuters, respectively. In 12 nonsmoking male car repair workers, blood benzene levels ranged from 0.11 to 0.98 μg/L (median=0.19 μg/L); in 8 smoking male car repair workers, levels ranged from 0.17 to 0.67 μg/L (median=0.42 μg/L). Three nonsmoking male gasoline attendants had blood benzene levels ranging from 0.32 to 0.47 μg/L (median=0.36 μg/L) (White et al. 1993).

Pumping gasoline can also be a significant source of exposure to benzene. A study conducted between July 1998 and March 1999 that comprised of 39 customers of gasoline self service stations from North Carolina, measured the benzene level in the air around the station as well as the levels of benzene in customers' breath prior to and immediately after fueling (Egeghy et al. 2000). Benzene levels in the air around the station ranged from <0.02 to 11.16 ppm, with a mean (±1 standard deviation) of 0.91 (±1.8) ppm. The range of benzene levels in the breath of customers prior to fueling was <0.001–0.022 ppm with a mean (±1 standard deviation) of 0.0027 (±0.0034) ppm while the range of benzene levels in the breath of customers after re-fueling was <0.001–0.434 ppm with a mean (±1 standard deviation) of 0.05 (±0.081) ppm (Egeghy et al. 2000). Another study reported a benzene concentration of 1 ppm at the breathing-level of a person pumping gas (EPA 1986). Using this concentration and an estimated 70 minutes/year of time spent pumping gasoline, a benzene intake of 10 μg/day has been calculated (Wallace 1989a). In a group of 26 subjects who were not occupationally exposed to benzene, but were exposed to benzene during refueling in Fairbanks, Alaska, median blood benzene levels prior to and immediately following refueling were 0.19 ppb (range 0.08–0.65 ppb) and 0.54 ppb (range 0.13–1.70 ppb), respectively (Backer et al. 1997).

Gasoline vapors vented into the home from attached garages can also increase indoor air exposure to benzene (Wallace 1989a, 1989b). Depending on airflow from garage to living areas, mean indoor benzene concentrations in houses with a garage were 2–5 times higher than outdoor levels in most homes (Thomas et al. 1993). Benzene levels in four garages during different times in a day ranged from 0.94 to 61.3 ppb. The higher concentrations of benzene in these garages were not only from vehicular activity, but also in varying proportions from stored gasoline, paints, and benzene-containing consumer products (Thomas et al. 1993). Inhalation exposure to off-gassing from benzene-containing products and to evaporative emissions from automobiles in attached garages has been estimated to be 150 μg/day (Wallace 1989a).

A study of human exposure to benzene in the California South Coast Air Basin showed that benzene exposure in that area has decreased greatly since 1989. Adult smokers saw a 28% decrease in benzene exposure from 1989 to 1997, while adult nonsmokers and adolescents saw a decrease in benzene exposure of 67 and 55%, respectively. Where people were being exposed to benzene also changed during that time. Ambient air was the source of 49% of the benzene that nonsmokers were exposed to in 1997, which is less than the 59% from 1987. For smokers in the region, 85% of their benzene exposure was from smoking and 4% was from the ambient air in 1997. This shows an increase in benzene exposure from smoking since 1987 (78%) and a decrease in exposure from the ambient air (12%) (Fruin et al. 2001). The main sources credited with this decrease in exposure are reformulated gasoline and stricter air emission laws as well as smoking control measures.

Another source of exposure to benzene for the general population is the use of domestic wood stoves. It has been estimated that approximately 10% of the space heating in urban areas of the northern United States is from wood burning, with up to 50% in smaller, rural towns (Larson and Koenig 1994). Benzene has been found to be a major component of the emissions from wood burning, especially from efficient flame combustion, and constituted roughly 10–20% by weight of total non-methane hydrocarbons (Barrefors and Petersson 1995). It should be noted, however, that chimney emissions result in much lower human exposure than equally large emissions at the ground level.

Other sources of inhalation exposure to benzene include air around hazardous waste sites, industrial facilities, off-gassing from particle board, and off-gassing from contaminated water during showering and cooking. Based on the TEAM study findings, it appears that the following are not important sources of

exposure to benzene on a nationwide basis: chemical manufacturing facilities, petroleum refining operations, oil storage tanks, drinking water, food, and beverages (Wallace 1989a).

Average water intake of benzene (assuming a typical drinking water concentration of 0.1 ppb and a consumption of 2 L/day) is 0.2 μg/day (HSDB 2007). According to another estimate, the daily intakes of benzene for a nonsmoking individual (not exposed to secondary smoke) are 1–550 μg (Fishbein 1992).

While most human exposure to benzene is believed to be through inhalation, studies show that benzene can permeate skin with a permeability factor of about 0.14–0.18 cm/hour at 25 °C. The permeability factor was not affected by moisturizer, baby oil, or insect repellent; however, it was affected by temperature (50 °C) and sunscreen with the permeability factors increasing to 0.26 and 0.24 cm/hour, respectively (Nakai et al. 1997).

Individuals employed in industries that use or make benzene or products containing benzene may be exposed to the highest concentrations of benzene. The National Occupational Exposure Survey (NOES), conducted by NIOSH from 1981 to 1983, estimated that approximately 272,300 workers employed in various professions were potentially exposed to benzene in the United States. Approximately half of these workers were employed in general medical and surgical hospitals, and their occupations included nurses and aides, physicians, technicians, technologists, therapists, dieticians, pharmacists, and janitors (NIOSH 1990). The NOES database does not contain information on the frequency, concentration, or duration of exposure; the survey provides only estimates of workers potentially exposed to chemicals in the workplace. The current OSHA permissible limit for an 8-hour TWA exposure to benzene is 1 ppm and a short-term exposure limit in any 15-minute period is 5 ppm (OSHA 2003). The NIOSH recommended exposure limit is 0.1 ppm for an 8-hour TWA and 1 ppm for short-term exposure (NIOSH 1992b). In 1987, OSHA estimated that approximately 238,000 workers were exposed to benzene in seven major industry sectors, including petrochemical plants, petroleum refineries, coke and coal chemicals, tire manufacturers, bulk terminals, bulk plants, and transportation via tank trucks (Table 6-4) (OSHA 1987). Approximately 10,000 workers were estimated to be exposed to TWA concentrations in excess of the 1 ppm standard. This estimate did not include firms covered by the exclusions, firms under jurisdiction of other agencies, or firms involved in the use of products containing small quantities of benzene. The uptake of benzene by workers in a municipal waste incinerator in Germany was assessed by measuring benzene levels in blood (Angerer et al. 1991). No significant difference ($p<0.05$) in blood benzene levels between workers and controls were detected (mean 0.22 μg/L for nonsmoking workers versus 0.25 μg/L

Table 6-4. Percentage of Employees Exposed to Benzene by Exposure Level and Industry Division[a]

Industry sector	Percentage of observations in each exposure category according to range of 8-hour TWA benzene concentrations (ppm)						Total number of employees
	0.0–0.1	0.11–0.5	0.51–1.0	1.1–5.0	5.1–10	10+	
Petrochemical plants[b]		74.6		23.0	2.4	0.0	4,300
Petroleum refineries[c,d]	64.6	26.1	4.6	3.8	0.5	0.4	47,547
Coke and coal chemicals[e]	0.0	39.3	27.6	27.5	4.4	1.3	947[f]
Tire manufacturers[c]	53.4	37.5	6.3	2.8	0.0	0.0	65,000
Bulk terminals[c]	57.8	32.8	5.3	3.7	0.3	0.1	27,095
Bulk plants[c]	57.8	32.8	5.3	3.7	0.3	0.1	45,323
Transportation via tank truck[c]	68.4	23.1	5.3	2.9	0.1	0.2	47,600
Total							237,812

[a]Derived from OSHA 1987
[b]Percentages represent the portion of workers whose average exposures are in each category.
[c]Percentages represent the portion of sampling results in each category.
[d]Data do not reflect respirator use and sampling biases.
[e]Percentages represent the portion of workers whose average exposures are in each category.
[f]Excludes workers employed at the coke ovens.

TWA = time-weighted average

for nonsmoking controls). OSHA requires the use of engineering controls and/or respiratory protection in situations where compliance with the TWA is not feasible (OSHA 1987).

Certain jobs, such as gasoline station workers, firefighters, and dry cleaners, are believed to put people at a higher risk of benzene exposure. In an analysis of literature, it was estimated that workers in the area of crude petroleum and natural gas are exposed to 0.04 ppm benzene, while workers in petroleum refining, gas stations, and crude petroleum pipelines are exposed to 0.22, 0.12 and 0.25 ppm benzene, respectively. This study also showed that fire fighters are exposed to an average of 0.38 ppm benzene (van Wijngaarden and Stewart 2003). Workers from four different dry cleaning facilities in Korea had mean benzene air concentrations ranging from 2.7 to 3.2 ppb. Their exposure to benzene was dependent upon the type of solvent used for cleaning (Jo and Kim 2001). Benzene concentrations of 25.46 and 1,331.29 ppb were found near the kiln and at the rotary line, respectively, inside a hazardous waste incinerator in Turkey (Bakoglu et al. 2004).

A study comparing workers who were exposed to benzene regularly at work to people who were not exposed to benzene at work showed that while the general population in Italy had average blood benzene concentration of 165 ng/L, the people who were exposed to high benzene levels at work had an average benzene blood concentration of 186 ng/L. Immediately following their shift, the average benzene blood level samples from of benzene-exposed workers was 420 ng/L. The average blood benzene levels for smoking and nonsmoking occupationally exposed workers were 264 and 123 ng/L respectively (Brugnone et al. 1998).

6.6 EXPOSURES OF CHILDREN

This section focuses on exposures from conception to maturity at 18 years in humans. Differences from adults in susceptibility to hazardous substances are discussed in Section 3.7, Children's Susceptibility.

Children are not small adults. A child's exposure may differ from an adult's exposure in many ways. Children drink more fluids, eat more food, breathe more air per kilogram of body weight, and have a larger skin surface in proportion to their body volume. A child's diet often differs from that of adults. The developing human's source of nutrition changes with age: from placental nourishment to breast milk or formula to the diet of older children who eat more of certain types of foods than adults. A child's behavior and lifestyle also influence exposure. Children crawl on the floor, put things in their mouths,

sometimes eat inappropriate things (such as dirt or paint chips), and spend more time outdoors. Children also are closer to the ground, and they do not use the judgment of adults to avoid hazards (NRC 1993).

Children can be subject to increased benzene exposure by inhalation of second-hand smoke. In a study of nonsmoking rural families, urban families, and urban smoking families, infant exposure to benzene was estimated at doses of 15.3, 19.7, and 25.9 μg/day, respectively, with daily intakes of 1.68, 2.16, and 2.55 μg/kg body weight/day, respectively. For children of the same classification, benzene exposure was measured at doses of 29.3, 37.6, and 49.3 μg/day, respectively, with daily intakes of 0.71, 0.91, and 1.20 μg/kg bodyweight/day, respectively. For all infants and children, benzene exposure predominantly comes from the indoors (Duarte-Davidson et al. 2001).

Depending on a child's living environment, they may have higher exposure to benzene than adults. In a study of two lower-income areas of Minneapolis, children were found to have average personal benzene exposures of 0.66 and 0.53 ppb in the winter and spring, respectively. The highest concentration of benzene in their environment came from the home with winter and spring concentrations of 0.69 and 0.66 ppb respectively, while the outdoor and school benzene concentrations were 0.41 and 0.19 ppb, respectively (Adgate et al. 2004). In Italy, concentrations of the benzene metabolite, *trans,trans*-muconic acid (MA), was measured in the urine of children from both urban areas in Naples and rural areas in Pollica. The mean urinary concentrations of MA detected for rural and urban children were 48.4 and 98.7 μg/L (Amodio-Cocchieri et al. 2001). These studies also found no strong link between passive smoking and MA levels. The only factor that affected levels of MA in urine samples was how close the family lived to the road. A study in Rouen, France, compared benzene exposure and concentrations in nonsmoking parents and their children. Despite the fact that the children were exposed to slightly less benzene 3.47 ppb (11.1 $\mu g/m^3$) than their parents 4.51 ppb (14.4 $\mu g/m^3$), there was no significant correlation between exposure means and urinary metabolite levels (Kouniali et al. 2003).

Benzene concentrations in women and their tissue as well as breast milk have been a major concern. In a study of South Korean housewives living near service stations, indoor, outdoor and breath benzene concentrations averaged 5.73, 3.63, and 3.29 ppm, respectively (Jo and Moon 1999). Benzene has been found in mother's milk (Pellizzari 1982). Benzene was found at mean concentrations of 0.06 μg/kg in 23 samples of breast milk taken from a children's hospital in Rome (Fabietti et al. 2004). While this may provide a mechanism by which infants are exposed to benzene, these concentrations are lower than in other foods.

6.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Individuals who live near hazardous waste sites or near leaking underground fuel storage tanks might be exposed to potentially high concentrations of benzene in their drinking water if they obtain tap water from wells located near these sources. In a series of experiments conducted in a single-family residence from June 11 to 13, 1991, exposure to benzene through contaminated residential water was monitored (Lindstrom et al. 1994). The residential water was contaminated with benzene and other hydrocarbons in 1986. Periodic testing conducted from 1986 to 1991 showed benzene concentrations ranging from 33 to 673 μg/L (ppb). The experiment involved an individual taking a 20-minute shower with the bathroom door closed, followed by 5 minutes for drying and dressing; then the bathroom door was opened and this individual was allowed to leave the house. Integrated 60- and 240-minute whole-air samples were collected from the bathroom, an adjacent bedroom, living room, and in ambient air. Glass, gas-tight syringe grab samples were simultaneously collected from the shower, bathroom, bedroom, and living room at 0, 10, 18, 20, 25, 25.5, and 30 minutes. Two members of the monitoring team were measured for 6 hours using personal Tenax gas GC monitors. For the first 30 minutes of each experiment, one member was based in the bathroom and the other in the living room. Benzene concentrations in the shower head ranged from 185 to 367 μg/L (ppb), while drain level samples ranged from below the detectable limit (0.6 μg/L or ppb) to 198 μg/L (ppb). Analysis of the syringe samples suggested a pulse of benzene moving from the shower stall to the rest of the house over approximately 60 minutes. Peak benzene levels were measured in the shower stall at 18–20 minutes (758–1,670 $\mu g/m^3$), in the bathroom at 10–25 minutes (366–498 $\mu g/m^3$), in the bedroom at 25.5–30 minutes (81–146 $\mu g/m^3$), and in the living room at 36–70 minutes (40–62 $\mu g/m^3$). The total benzene dose resulting from the shower was estimated to be approximately 281 μg, with 40% via inhalation and 60% via the dermal pathway (Lindstrom et al. 1994).

The major source of exposure to benzene is cigarette smoke. A smoker of 32 cigarettes per day (the U.S. average per smoker) would have a benzene intake of approximately 1.8 mg/day (at least 10 times the average nonsmoker's intake) (Wallace 1989a). Median benzene concentrations in 343 homes with smokers averaged 3.3 ppb compared to 2.2 ppb in 185 homes without smokers. This represents a 50% increase in benzene exposure for nonsmokers exposed to passive smoke compared to nonsmokers not exposed to passive smoke (Wallace 1989a). In a study in Germany, the mean benzene concentrations for frequent smokers and nonsmokers were 6.1 and 2.4 ppb, respectively (Hoffmann et al. 2000).

6.8 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of benzene is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of benzene.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.8.1 Identification of Data Needs

Physical and Chemical Properties. The physical and chemical properties of benzene are well characterized and allow prediction of the transport and transformation of the compound in the environment.

Production, Import/Export, Use, Release, and Disposal. According to the Emergency Planning and Community Right-to-Know Act of 1986, 42 U.S.C. Section 11023, industries are required to submit substance release and off-site transfer information to the EPA. The TRI, which contains this information for 2004, became available in May of 2006. This database is updated yearly and should provide a list of industrial production facilities and emissions.

Benzene is one of the top 20 highest volume chemicals produced in the United States. In 1994, the U.S. production volume of benzene was 14.7 billion pounds (C&EN 1995). The production volume during the 1984–1994 period has increased by 4% annually (C&EN 1995). The United States currently has a benzene production capacity of 11.8 billion liters (SRI 2004). Imports of benzene into the United States have generally ranged from 10,176 to 11,672 million pounds during 2002–2004 (USITC 2005). Exports increased from 21 million pounds in 2002 to 290 million pounds in 2003, but decreased to 145 million pounds in 2004 (USITC 2005). The major use of benzene is in the production of other chemicals (primarily ethylbenzene, cumene, and cyclohexane), accounting for approximately 91% of benzene

production volume. Benzene is also used in chemical laboratories as a solvent and a reactant (OSHA 1977, 1987), and as an anti-knock agent in unleaded gasoline (Brief et al. 1980; EPA 1985a). The widespread use of benzene as a solvent has decreased in recent years. Many products that used benzene as a solvent in the past have replaced it with other organic solvents; however, benzene may still occur as a trace impurity in these products. Less than 2% of the amount of benzene produced is used as a solvent in such products as trade and industrial paints, rubber cements, adhesives, paint removers, artificial leather, and rubber goods. Benzene has also been used in the shoe manufacturing and rotogravure printing industries (EPA 1978; OSHA 1977). In the past, certain consumer products (such as some paint strippers, carburetor cleaners, denatured alcohol, and rubber cement used in tire patch kits and arts and crafts supplies) contained small amounts of benzene (Young et al. 1978). Other consumer products that contained benzene were certain types of carpet glue, textured carpet liquid detergent, and furniture wax (Wallace et al. 1987). The use of benzene in certain pesticides has been canceled. Benzene-containing wastes, such as commercial chemical products, manufacturing chemical intermediates, and spent solvents, are subject to federal and/or state hazardous waste regulations (HSDB 2007). Currently, the recommended method of disposal is to incinerate solvent mixtures and sludges at a temperature that ensures complete combustion. No additional information on the production, import/export, use, release, or disposal of benzene is needed at this time.

Environmental Fate. Benzene released to the environment partitions mainly to the atmosphere (Mackay and Leinonen 1975). However, the compound can also be found in surface water and groundwater. Benzene is mobile in soil (Karickhoff 1981; Kenaga 1980); however, there is a need for more information on the leachability potential of benzene to groundwater in different soil types. Benzene is transformed in the atmosphere by photooxidation. Biodegradation, principally aerobic, is the most important fate process of benzene in water (Delfino and Miles 1985; McAllister and Chiang 1994; Salanitro 1993) and soil (Gibson 1980; Hopper 1978; Salanitro 1993). Benzene can persist in groundwater. No additional information on the environmental fate of benzene is needed at this time.

Bioavailability from Environmental Media. Benzene can be absorbed following oral exposure (Thienes and Haley 1972), dermal exposure (Blank and McAuliffe 1985; Franz 1984; Laitinen et al. 1994; Lindstrom et al. 1994; Lodén 1986; Susten et al. 1985), and inhalation exposure (Ashley et al. 1994; Avis and Hutton 1993; Boogaard and van Sittert 1995; Brunnemann et al. 1989; Byrd et al. 1990; Etzel and Ashley 1994; Fustinoni et al. 1995; Ghittori et al. 1995; Gordian and Guay 1995; Hajimiragha et al. 1989; Hanzlick 1995; HazDat 2006; Karacic et al. 1995; Kok and Ong 1994; Lagorio et al. 1994a; Laitinen et al. 1994; Lauwerys et al. 1994; Lindstrom et al. 1994; Mannino et al. 1995; Nomiyama and

Nomiyama 1974a; Ong and Lee 1994; Ong et al. 1995; Pekari et al. 1992; Popp et al. 1994; Rauscher et al. 1994; Rothman et al. 1995; Ruppert et al. 1995; Scherer et al. 1995; Shamy et al. 1994; Srbova et al. 1950). These routes of exposure may be of concern to humans because of the potential for benzene to contaminate the air (Bennett 1987; Black et al. 1980; Brief et al. 1980; Cal EPA 1987; Edgerton and Shah 1992; EPA 1989, 1994d; Glass et al. 1986; Graedel 1978; Mayer et al. 1994; TRI02 2005; Wallace 1989a, 1989b; Wallace and Pellizzari 1986; Wester et al. 1986; Wood and Porter 1987), drinking water (CDC 1994; EPA 1979), and soil (HazDat 2006; Mushrush et al. 1994; TRI02 2005). Information on inhalation exposure and on the absorption of benzene following ingestion of plants grown in contaminated environments near hazardous waste sites would be helpful in determining bioavailability of the compound in these media.

Food Chain Bioaccumulation. Benzene has an estimated low-to-moderate bioconcentration potential in aquatic organisms (Miller et al. 1985; Ogata et al. 1984) and some plants (Geyer et al. 1984). Most of the benzene accumulation on vegetation results from air-to-leaf transfer. Root uptake is not believed to be important (Hattemer-Frey et al. 1990). Biomagnification in aquatic food chains does not appear to be important (Ogata et al. 1984). No further information appears to be needed.

Exposure Levels in Environmental Media. Reliable monitoring data for the levels of benzene in contaminated media at hazardous waste sites are needed so that the information obtained on levels of benzene in the environment can be used in combination with the known body burden of benzene to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

Benzene is widely distributed in the environment and has been detected in air (Clayton et al. 1999; EPA 1987a; Mohamed et al. 2002; Morello-Frosch et al. 2000), water (EPA 2001, 2005f; Sauer 1981, USGS 2005), soil (EPA 1979; Ferrario et al. 1985; MacLeod and MacKay 1999; Staples et al. 1985), sediment, and some foods (EPA 1980b, 1982a; Fleming-Jones and Smith 2003). The levels of benzene in air and water are well documented, but there is a need for more current information. Benzene is not expected to be a significant contaminant in aquatic foods (Geyer et al. 1984; Gossett et al. 1983; Miller et al. 1985; Ogata et al. 1984); however, some contamination of food crops consumed by humans may occur, primarily from air-to-leaf transfer (Hattemer-Frey et al. 1990). The total concentration of benzene on exposed food crops consumed by humans was estimated to be 587 ng/kg (Hattemer-Frey et al. 1990). Humans are at risk of exposure to benzene because of its widespread distribution in the environment, particularly in the atmosphere. Releases to the air from gasoline, smoking, and automobile exhaust constitute the major risk of potential exposure for the general population (Wallace 1995). Additional data

characterizing the concentration of benzene in drinking water, air, and soil surrounding hazardous waste sites would be helpful in assessing human exposure for populations living near these waste sites. In addition, more current data on levels of benzene in foods would be helpful in estimating intake of benzene from food.

Exposure Levels in Humans. Benzene has been detected in human body fluids and tissues such as blood, urine, and fat (Brugnone et al. 1989; Chao et al. 1993; Karacic et al. 1987). Most of the monitoring data have come from occupational studies of specific worker populations exposed to benzene (Inoue et al. 1989b; Karacic et al. 1987; OSHA 1987; van Wijngaarden and Stewart 2003). Biological monitoring studies exist for the general population (Melikian et al. 1994). There is information for background levels in breath of smokers and nonsmokers (Wallace 1989b), baseline blood levels (Karacic et al. 1987), and levels of urinary metabolites in unexposed people (Inoue et al. 1989b). Information on exposure levels for populations living in the vicinity of hazardous waste sites would be helpful in estimating exposure in these groups.

This information is necessary for assessing the need to conduct health studies on these populations.

Exposures of Children. Benzene levels have been monitored in children and the environments in which they live. This information gives levels found for infants and children in rural and urban area as well as the levels found for children in homes of parents who smoke (Duarte-Davidson et al. 2001). There have been many studies relating oil and petroleum exposure to childhood leukemia and other diseases; however, the majority of these studies have not recorded benzene levels. More information about the exposures of children, particularly those subject to high exposures such as smoking, busy roads, and gasoline stations, are needed.

Child health data needs relating to susceptibility are discussed in Section 3.12.2, Identification of Data Needs: Children's Susceptibility.

Exposure Registries. In 2001, 1,143 people were included in the benzene subset of the Volatile Organics Compounds subregistry of the National Exposure Registry. These people were exposed to benzene at a site in Texas. Demographic and health information was obtained on all the exposed persons; the information will be updated longitudinally. For those who were identified as exposed and were deceased, a death certificate will be obtained to ascertain cause of death. This activity was carried out by the Exposure and Disease Registry Branch (EDRB), Division of Health Studies (DHS), ATSDR. The

data became part of public-user data files maintained by ATSDR. The statistical analyses of the baseline data was completed and published (Agency for Toxic Substances and Disease Registry 1995). The information that was amassed in the National Exposure Registry will be used to facilitate the epidemiological research needs to assess adverse health outcomes that may be related to the exposure to this substance.

6.8.2 Ongoing Studies

The Federal Research in Progress (FEDRIP 2005) database provides additional information obtainable from a few ongoing studies that may fill in some of the data needs identified in Section 6.8.1. These studies are summarized in Table 6-5.

Table 6-5. Ongoing Studies on the Potential for Human Exposure to Benzene[a]

Investigator	Affiliation	Research description	Sponsor
Cho, CY	CHA Corporation; Laramie, Wyoming	Study of the use of microwave technology for superfund site remediation	NIEHS
Fischer, LJ		Understanding potential health hazards from groundwater and soil contamination of chemicals commonly found at hazardous waste sites	USDA
Greenberg, A		Study of critical steps in the ring-opening metabolism of the human carcinogen benzene to muconaldehyde	USDA
Gurian, PL	University of El Paso Texas; El Paso, Texas	Modeling of organic compounds in drinking water	National Institute of General Medical Sciences
Nylander-French, LA	University of North Carolina; Chapel Hill, North Carolina	Assessment of dermal exposure to benzene and naphthalene	NIEHS
Rothman, N		Study of occupational and environmental exposures	Division of cancer epidemiology and genetics
Scow, KM	University of California; Davis, California	Molecular characterization of aquifer microbial communities	NIEHS
Stenzel, PS	National Institute of Health	Study of the link between occupational exposure of carcinogens and cancer	Division of cancer epidemiology and genetics
Thrall, KD	Oregon Health and Science University; Portland, Oregon	Assessment of human volatile organic compounds exposure near Superfund sites	NIEHS
Weisel, CP		Study of the modulation of benzene metabolism by exposure to environmental mixtures	USDA

[a]FEDRIP 2005

NIEHS = National Institute of Environmental Health Sciences; USDA = U.S. Department of Agriculture

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7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring benzene, its metabolites, and other biomarkers of exposure and effect to benzene. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision.

7.1 BIOLOGICAL MATERIALS

Analytical methods have been developed to measure benzene levels in exhaled breath, blood, and various body tissues. The primary method of analyzing for benzene in exhaled breath, body fluids, and tissues is gas chromatography (GC) coupled with either flame ionization detection (FID), photoionization detection (PID), or mass spectrometry (MS). Rigorous sample collection and preparation methods must be followed when analyzing for benzene to prevent contamination of the sample. A summary of commonly used methods of measuring benzene in biological samples is presented in Table 7-1.

Breath samples are collected on a solid sorbent (EPA 1987b; Gruenke et al. 1986; Pellizzari et al. 1988; Wallace et al. 1985), in canisters (Thomas et al. 1991), or in a breath sampling tube and analyzed directly (Sherwood and Carter 1970). Samples collected on Tenax sorbent are subjected to a thermal desorption/cryofocussing step prior to analysis by capillary GC/MS (EPA 1987b; Pellizzari et al. 1988; Wallace et al. 1985). Techniques involving headspace analysis of benzene adsorbed on silica gel have also been used (Gruenke et al. 1986). MS detection generally provides the most sensitivity, from the low to sub-ppb. The selectivity of the methods is improved if capillary GC columns are used (Pellizzari et al. 1988). Extraction of benzene from blood is frequently accomplished by either purge-and-trap or headspace analysis. In purge-and-trap analysis, an inert gas such as helium or nitrogen is passed through the sample, and purged volatiles are trapped on an appropriate solid sorbent (Antoine et al. 1986; Ashley et al. 1992, 1994; Michael et al. 1980). Recent improvements in the method have resulted in excellent sensitivity (300 ppt) and acceptable precision and accuracy (Ashley et al. 1992, 1994). The purge-and-trap method has also been used to analyze breast milk for other volatile organic compounds and could be

Table 7-1. Analytical Methods for Determining Benzene in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Breath	Collection on Tenax GC; thermal desorption	HRGC/MS (IARC Method 5)	3 ppt	70–130 estimated	Pellizzari et al. 1988
Breath	Collection in sampling tube; direct injection of sample	GC/FID	100 ppb	100	Sherwood and Carter 1970
Breath	Collection on Tenax GC; thermal desorption to on-column cryogenic trap	HRGC/MS	1.6 ppb (5-L sample)	86–90	Wallace et al. 1986, 1985
Breath	Collection in bags, adsorption on silica gel; desorption to headspace vial; analysis of headspace gases	GC/MS-SIM	0.1 ppb	NR	Gruenke et al. 1986
Blood	Purge and trap	HRGC/MS	30 ppt	112–128	Ashley et al. 1992; 1994
Blood	Heparinization; transfer to isotonic saline in headspace vial; equilibration with heat	HRGC/PID	0.4 μg/L	NR	Pekari et al. 1989
Blood	Collection and transfer to headspace vial; analysis of headspace gases	GC/MS-SIM	2 μg/L	NR	Gruenke et al. 1986
Blood	Purging with nitrogen; collection on Tenax GC-silica gel	GC/MS	0.5 μg/L	NR	Antoine et al. 1986
Blood	Extraction with toluene + HCl; centrifugation; analysis of toluene layer	GC/FID	100 μg/L	98–100	Jirka and Bourne 1982
Blood	Extraction device coupled to MS	ITMS	90 ppt	NR	St-Germain et al. 1995
Blood	Centrifugation; dialysis; precipitation; acidic hydrolysis; Sep Pak cartridge column purification	HPLC	20 pmol/g globin	NR	Hanway et al. 2000
Urine	Incubation; analysis of headspace gases	GC/PID	0.51 nmol/L	>90	Kok and Ong 1994
Tissues (bone marrow, fat)	Homogenization with internal standard; centrifugation; analysis of supernatant	GC/MS-SIM	NR	NR	Rickert et al. 1979
Tissues (lung, liver)	Homogenization in buffer; centrifugation; analysis of supernatant	RID-preparative HPLC/UV	20 pg/g	NR	Bechtold et al. 1988

FID = flame ionization detection; GC = gas chromatography; HCl = hydrochloric acid; HPLC = high-performance liquid chromatography; HRGC = high resolution gas chromatography; IARC = International Agency for Research on Cancer; ITMS = ion trap mass spectrometry; MS = mass spectrometry; NR = not reported; PID = photoionization detection; RID = reverse isotope dilution; SIM = selected ion monitoring; UV = ultraviolet detection

used for analyzing benzene (Michael et al. 1980; Pellizzari 1982). For headspace analysis, the samples are placed in a special vial, and the gas generated above the liquid sample under equilibrium conditions is analyzed (Gruenke et al. 1986; Pekari et al. 1989). Sensitivity is in the sub- to low-ppb range. A third method of sample preparation involves extraction of the blood sample with an organic solvent (Jirka and Bourne 1982) and analysis of the organic fraction. These methods are generally less sensitive, with reported detection limits usually in the low- to mid-ppb range. Selectivity is improved with use of high resolution gas chromatography (HRGC). Accuracy and precision could not be adequately compared given the limited data available.

Screening methods are available for analysis of benzene in feces and urine (Ghoos et al. 1994) and body fluids (Schuberth 1994). Both employ analysis by capillary GC with an ion trap detector (ITD). Benzene in urine has been determined by trapping benzene stripped from the urine on a Carbotrap tube, followed by thermal desorption GC/FID. Care must be taken when preparing benzene metabolite samples from urine and bodily fluids in order to protect against enzymatic and oxidative degradation. These samples are often treated to denature enzymes and avoid oxidation by hydroquinone. The detection limit is 50 ng/L and the average recovery is approximately 82% (Ghittori et al. 1993). Benzene in urine has also been determined using headspace analysis with capillary GC/PID. The detection limit is 40 ng/L (Kok and Ong 1994).

Methods are also available for determining metabolites of benzene in urine. A summary of available methods is shown in Table 7-2. Both GC/FID or GC/MS and high-performance liquid chromatography (HPLC) with ultraviolet detection (UV) have been used to measure urinary metabolites.

The primary metabolite of benzene is phenol. Phenol is excreted as glucuronide and sulphate conjugates in urine. Total phenolic metabolites (phenol, phenyl sulfate, and phenyl glucuronide) have been determined by hydrolyzing urine samples either enzymatically or by acid, then extracting the phenol with solvent. Phenol is then measured by GC or HPLC techniques. Enzymatic hydrolysis coupled with GC/FID has been reported; the detection limit is 1 mg/L and recovery is excellent (92–98%) (IARC 1988). Sulfate and glucuronide conjugates have been determined directly by HPLC/UV (Ogata and Taguchi 1987). The normal baseline levels of urinary phenolic metabolites from humans are usually 2–18 mg/L (Ong and Lee 1994). The available methods are sensitive enough to measure these relatively high amounts accurately.

Table 7-2. Analytical Methods for Determining Metabolites of Benzene in Urine

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine (phenol, phenyl sulfate, phenyl glucuronide)	Centrifugation	HPLC/UV	4 mg/L (PS); 5 mg/L (PG)	100.5 (PS); 101.8 (PG)	Ogata and Taguchi 1987
Urine (phenol, phenyl sulfate, phenyl glucuronide)	Digestion (enzymatic and with acid); extraction with diethyl ether	GC/FID (IARC Method 6)	1 mg/L	92–98	IARC 1988
Urine (phenols and cresols)	Hydrolysis with perchloric acid; extraction with diisopropyl ether	GC/FID	NR	NR	NIOSH 1974
Urine (phenols and cresols)	Hydrolysis with perchloric acid; saturation with NaCl; extraction with diisopropyl ether	GC/FID	2 mg/L	NR	Roush and Ott 1977
Urine (phenol)	Enzymatic reaction	HPLC/fluoro-metric detection	50 ppb	97.7	Jen and Tsai 1994
Urine (*trans,trans*-muconic acid)	Mixing with methanol; centrifugation	HPLC/UV	0.1 mg/L	NR	Inoue et al. 1989
Urine (muconic acid, phenol)	Mixing with formic acid; extraction (twice) with ethyl ether; evaporation of combined extracts	GC/MS	10 μg/L	NR	Bechtold et al. 1991
Urine (*trans,trans*-muconic acid)	Cleanup on anion exchange resin; derivitization	GC/MS	0.01 mg/L	93–106	Ruppert et al. 1995
Urine (S-phenyl-mercapturic acid	Solid-phase extraction; hydrolysis; derivatization	HPLC	1 μg/L	NR	Einig and Dehnen 1995
Urine (*trans,trans*-muconic acid)	Cleanup on anion exchange resin	HPLC/UV	5 ppb	NR	Weaver et al. 2000

FID = flame ionization detection; GC = gas chromatography; HPLC = high performance liquid chromatography; HRGC = high resolution gas chromatography; IARC = International Agency for Research on Cancer; MS = mass spectrometry; NaCl = sodium chloride; NR = not reported; PG = phenyl glucuronide; PS = phenyl sulfate; UV = ultraviolet detection

Analysis of urinary *trans,trans*-muconic acid seems to be a better indicator than phenol for assessing exposure to low levels of benzene (Ducos et al. 1990). However, muconic acid is a minor metabolic route and background levels of muconic acid in urine are much lower than levels of phenolic metabolites and are frequently below the limit of detection of the method used to determine them (Inoue et al. 1989b). The detection of low levels of *trans,trans*-muconic acid in urine was difficult by earlier methods because of low recovery of *trans,trans*-muconic acid (37% with ether) by the commonly used solvent extraction method (Gad-El-Karim et al. 1985). An improved method for the determination of urinary *trans,trans*-muconic acid utilizes solid phase extraction with SAX sorbent in combination with the HPLC/UV for quantitation. The detection limit is 0.06–0.1 mg/L, and recovery is very good (90%) (Boogaard and van Sittert 1995; Ducos et al. 1990). Weaver et al. (2000) used an anion exchange column to extract the urine sample, which was then analyzed using HPLC-UV; detection limits were around 5 ppb. The relative standard deviation of the method was 5% in the concentration range 1–20 ng/L. *trans,trans*-muconic acid has been determined directly by HPLC/UV with similar sensitivity (detection limit=0.1 mg/L) (Inoue et al. 1989b). The detection limit and specificity for the determination of urinary *trans,trans*-muconic acid may be improved by using HPLC with diode array detector, GC/FID of the methylated product, or GC/MS of trimethylsilyated product (Bartczak et al. 1994). Both GC/FID and HPLC/diode array detection are capable of detecting urinary *trans,trans*-muconic acid at concentrations above 40 μg/L, but GC/MS is capable of detecting the metabolite at concentrations below 40 μg/L (Bartczak et al. 1994).

The metabolite, S-phenyl-mercaptic acid, may be an indicator of exposure to benzene. It can be detected at low levels (1 μg/L) in urine using solid phase extraction and determination by HPLC (Einig and Dehnen 1995). After purification by reverse phase cartridge chromatography, S-phenyl cysteine was detected with sensitivity of about 20 pmol/g globin using HPLC (Hanway et al. 2000).

7.2 ENVIRONMENTAL SAMPLES

Methods exist for determining benzene in air (ambient, occupational, and industrial), water, sediment, soil, foods, cigarette smoke, gasoline, and jet fuel. Most involve separation by GC with detection by FID, PID, or MS. HPLC/UV and spectrophotometry have also been used. Table 7-3 summarizes several of the methods that have been used to analyze for benzene in environmental samples.

Numerous methods exist for detecting and measuring benzene in ambient air. Air samples for benzene analysis may be preconcentrated by passing the sample through a trap containing a solid adsorbent (Bayer et al. 1988; EPA 1979, 1980a; Fung and Wright 1986; Gruenke et al. 1986; Harkov et al. 1985; Reineke

Table 7-3. Analytical Methods for Determining Benzene in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Sample trapped on silica gel; thermal desorption	GC/MS	0.1 ppb	88–105	Gruenke et al. 1986
Air	Cryogenically trap; thermal desorption	GC/FID	NR	85–115	Singh et al. 1985
Air	Direct on-line analysis	GC/FID	NR	NR	Bayer et al. 1988
Air (ambient)	Direct injection of ambient air	GC/PID	0.25 ppb	NR	Clark et al. 1984
Air (ambient)	Direct analysis of ambient air	Electrochemical	NR	NR	Stetter et al. 1986
Air (ambient)	Sample collection in Tedlar bag; Cryogenically trap; thermally desorb	GC/PID	0.5 ppb	NR	Kowalski et al. 1985
Air (ambient)	Sample collection in stainless steel canisters or sorbent tubes; trap cryogenically; thermal desorption	HRGC/PID or HRGC/FID	5 ppt (PID) 24 ppt (FID)	97–104 (PID) 96–104 (FID)	Reineke and Bächmann 1985
Air (ambient)	Collect on charcoal (vapor badge or tube); desorb with carbon disulfide	GC/FID	0.3 ppb (estimated)	NR	Fung and Wright 1986
Air (ambient)	Cryogenically trap; thermal desorption	GC/PID/FID	1 ppt	70–130	Nutmagul and Cronn 1985
Air (ambient)	Collection in canisters; preconcentration using 2-stage trap	HRGC/ITD	sub-ppb level	8–13% bias	Kelly et al. 1993
Air (ambient)	Direct analysis	ALMS	250 ppb	NR	EPA 1985d
Air (ambient)	Sample collection onto on-column cryogenic sample loop or into stainless steel containers	GC/PID/FID	1 ppt	70–130	Nutmagul and Cronn 1985
Air (ambient)	Sample trapped on Tenax GC; thermal desorption to on-column cryogenic trap	HRGC	<0.1 ppb	69–126	EPA 1979
Air (ambient)	Sample collected on Tenax GC; thermal desorion to on-column cryogenic trap	GC/FID	0.03 ppb	56–144	EPA 1980a
Air (ambient)	Sample trapped on Tenax GC; thermal desorption to on-column cryogenic trap	HRGC/FID; conf. by HRGC/MS	3 ppt	NR	Roberts et al. 1984
Air (consumer products)	Sample trapped on solid sorbent; thermal desorption	GC/MS	NR	NR	Bayer et al. 1988

Table 7-3. Analytical Methods for Determining Benzene in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air (at waste sites and landfills)	Sample trapped on Tenax GC; thermal desorption	HRGC/FID/ECD; conf. by HRGC/MS	0.05 ppb	NR	Harkov et al. 1985
Air (occupational)	Sample trapped on charcoal; desorption with carbon disulfide	GC/FID (NIOSH Methods 1500 and 1501)	10–100 ppb	NR	NIOSH 1984
Air (occupational)	Sample collection on silica gel; desorption with ethanol	GC/FID	100 ppb	90	Sherwood and Carter 1970
Air (occupational)	Sample collection in Tedlar bag; direct injection	GC/PID (NIOSH Method 3700)	10 ppb	NR	NIOSH 1994
Air (occupational)	Sample collection on charcoal disk in miniature passive dosimeter; thermal desorption	GC/PID	60 ppb	85–115	Gonzalez and Levine 1986
Air (occupational; jet fuel fumes)	Sample collection on charcoal; desorption with methyl chloride-ethyl acetate	HPLC/UV	0.08 ppm	94–112	Dibben et al. 1989
Soil air	Sample collection on activated charcoal; desorption with carbon disulfide	GC/FID	NR	97–100	Colenutt and Davies 1980
Drinking water	Purge and trap	GC/MS	0.2 μg/L	NR	Brass et al. 1977
Drinking water	Purge and trap	HRGC/MS (EPA Method 524.2)	0.03–0.04 μg/L	97–99	EPA 1992a
Water	Purge and trap onto Tenax GC; thermal desorption to on-column cryogenic trap	HRGC/MS	0.1–10 μg/L	74–78	Michael et al. 1988
Water	Purge and trap on Tenax GC; thermal desorption to on-column cryogenic trap	HRGC	<1 μg/L	69–126	EPA 1979
Water	Purge and trap onto Tenax GC; thermal desorption	GC/MS	NR	85–125	Harland et al. 1985
Water	Solvent extraction with dichloromethane; concentration	GC/MS	15 μg/L	NR	Sporstøl et al. 1985
Water	Purge and trap on adsorbent column; thermal desorption	GC/PID (EPA Method 602)	0.2 μg/L	106	NEMI 2005a
Water	Purge and trap on activated carbon; desorption with carbon disulfide	GC/FID; conf. by GC/MS	NR	96–99	Colenutt and Thorburn 1980
Water	Purge and trap on Tenax; thermal desorption	GC/FID	0.001 μg/L	94–111	Hammers and Bosman 1986

Table 7-3. Analytical Methods for Determining Benzene in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Permeation of benzene through a silicone polycarbonate membrane into an inert gas stream	GC/FID	7.2 μg/L	NR	Blanchard and Hardy 1986
Waste water	Purge and trap onto adsorbent column; thermal desorption	GC/MS (EPA Method 624)	4.4 μg/L	113	NEMI 2005b
Waste water	Addition of isotopically labeled benzene analog; purge and trap onto adsorbent column; thermal desorption	GC/IDMS (EPA Method 1624)	10 μg/L	65–141	NEMI 2005c
Water, industrial effluents	Purge and trap on Tenax; thermal desorption	GC/MS	<5 μg/L	95–106	Pereira and Hughes 1980
Landfill leachate	Purge sample and trap on Tenax-silica gel; thermally desorb	GC/FID/FID	1 μg/L	NR	EPA 1984b
Landfill leachate	Extract sample with pentane	GC/MS	1,000–10,000 μg/L	NR	Schultz and Kjeldsen 1986
Solid wastes	Purge and trap, direct injection, vacuum distillation, or headspace	PID (EPA Method 8021B)	0.009 μg/L	99	EPA 1994c
Soil	Sample mixed with NaOH solution; equilibration; analysis of headspace gases	HRGC/FID; conf. by HRGC/MS	0.02 ng/mL	75–98	Kiang and Grob 1986
Soil	Purge and trap on Tenax; thermal desorption to on-column cryogenic trap	HRGC	<0.1 ppb	69–126	EPA 1979
Soil	Purge and trap on Tenax; thermal desorption	GC/FID	1 ppt	52	Hammers and Bosman 1986
Soil	Supercritical fluid extraction	HRGC/FID	low ppb	77–81	Burford et al. 1994
Sediment and biota	Purge and trap on Tenax GC-silica gel; thermal desorption	HRGC/MS	NR	NR	Ferrario et al. 1985
Sediment	Purge and trap on Tenax; thermal desorption	GC/MS	NR	64	Harland et al. 1985
Fruits and vegetables	Mix with water and methanol; filter; distill azeotrope	GC/FID	NR	84–96	Kozioski 1985
Shellfish	Tissue homogenized; purge with inert gas and trap on Tenax GC-silica gel; thermally desorb	GC/MS	NR	NR	Ferrario et al. 1985

Table 7-3. Analytical Methods for Determining Benzene in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Mainstream cigarette smoke	Collection on filters and impingers; [2H6]-benzene added to impinger	HRGC/IDMS-SIM	0.05 μg/ cigarette	75–85 (trapping efficiency)	Byrd et al. 1990
Cigarette smoke	Mainstream smoke filtered and analyzed directly; side-stream smoke and smoke-polluted air filtered and collected in cryogenic methanol-filled impingers	HRGC/MS-SIM	0.1 μg/ cigarette	NR	Brunnemann et al. 1989, 1990
Gasoline	Dilute sample with hexane	GC/FID	NR	NR	Poole et al. 1988
Gasoline	Dilute sample with methanol; elute benzene to analytical column with 50% methanol; back-flush guard column with 100% methanol	HPLC/UV	NR	NR	Ludwig and Eksteen 1988

ECD = electron capture detection; EPA = Environmental Protection Agency; FID = flame ionization detection; GC = gas chromatography; HPLC = high-performance liquid chromatography; HRGC = high resolution gas chromatography; IARC = International Agency for Research on Cancer; IDMS = isotope dilution mass spectrometry; ITD = ion trap mass spectrometry; LRS = laser Raman spectroscopy; MS = mass spectrometry; NaOH = sodium hydroxide; NIOSH = National Institute of Occupational Safety and Health; NR = not reported; PID = photoionization detection; SIM = selected ion monitoring; UV = ultraviolet detection

and Bächmann 1985; Roberts et al. 1984). Commonly used adsorbents are Tenax resins (e.g., Tenax TA, Tenax GC), silica gel, activated carbon, and carbonaceous polymeric compounds. Benzene in ambient air can be collected in stainless steel canisters (EPA 1988a; Kelly et al. 1993) or Tedlar bags (Kowalski et al. 1985) and can be analyzed with or without preconcentration. Preconcentration of benzene can be accomplished by direct on-column cryogenic trapping (EPA 1985c; Kowalski et al. 1985; Nutmagul and Cronn 1985; Reineke and Bächmann 1985; Singh et al. 1985), or samples may be analyzed directly without preconcentration (Bayer et al. 1988; Clark et al. 1984).

The most common methods of analysis for benzene in air are GC/PID, GC/FID, and GC/MS. The limit of detection for GC/FID and GC/PID ranges from low ppb to low ppt. GC/MS is generally considered to be more reliable than GC/FID or GC/PID in identifying benzene in samples, particularly those containing multiple components having similar GC elution characteristics. Benzene has been quantified in ambient air samples at sub-ppb levels by GC/MS (Gruenke et al. 1986) and ion trap mass spectrometry (Kelly et al. 1993). The ion trap detector has the advantage of remaining largely unaffected by water vapor in the sample. A continuous monitoring instrument using Fourier transform infrared (FTIR) spectroscopy is being developed for measuring the levels of benzene or other toxic chemicals in exhaust emissions from hazardous waste incinerators (DOE 1992).

Several analytical methods are available for determining atmospheric levels of benzene in the workplace. The OSHA recommended procedure involves the collection of the sample vapors on charcoal adsorption tubes, and then desorption followed by GC/MS analysis (OSHA 1985). Samples desorbed from charcoal are also analyzed by GC/FID (NIOSH 1984) or HPLC/UV (Dibben et al. 1989). Detection limits are in the ppb range (Dibben et al. 1989; NIOSH 1984). Passive dosimeters are also utilized, with GC/PID quantitation; detection limits are in the ppb range (Gonzalez and Levine 1986). Other acceptable methods include portable direct reading instruments and real-time continuous monitoring systems; these methods generally have a sensitivity in the ppm range.

The most frequently used analytical methods for water samples containing benzene are GC/MS, GC/FID, and GC/PID (Blanchard and Hardy 1986; Colenutt and Thorburn 1980; DOI 1984; EPA 1984, 1992; Hammers and Bosman 1986; Harland et al. 1985; Lysyj et al. 1980; Michael et al. 1988; Pereira and Hughes 1980; Sporstol et al. 1985). Benzene is usually isolated from aqueous media by the purge-and-trap method (Brass et al. 1977; Colenutt and Thorburn 1980; DOI 1984; EPA 1979, 1984, 1992; Hammers and Bosman 1986; Harland et al. 1985; Michael et al. 1988). An inert gas such as nitrogen is used to purge the sample. The purged benzene is trapped on an adsorbent substance, such as Tenax GC

or activated charcoal, and thermally desorbed. Recovery, where reported, ranges from acceptable (≈70%) (EPA 1979, 1984; Michael et al. 1988) to very good (≥90%) (Colenutt and Thorburn 1980; EPA 1984, 1992; Hammers and Bosman 1986). Detection limits in the sub-ppb to ppt range may be attained with HRGC/MS techniques (EPA 1992a); Michael et al. 1988). Liquid-liquid extraction procedures (Harrison et al. 1994; Schultz and Kjeldsen 1986; Sporstol et al. 1985) are less commonly used, having been replaced by more sensitive purge-and-trap methods. Interference from contamination can occur with all methods if extreme care is not used in the handling of samples and cleaning of all equipment.

Solid samples, such as soil, sediment, and foods, are most frequently prepared for analysis using the purge-and-trap method (EPA 1979, 1994c; Ferrario et al. 1985; Hammers and Bosman 1986; Harland et al. 1985), although supercritical fluid extraction has recently been utilized (Burford et al. 1994). Detection and quantitation of benzene may be GC/FID, GC/PID, or GC/MS. Detection limits as low as 1 ppt have been reported, but recoveries and precision have frequently been low. Improvements in the method, including analysis by HRGC/PID, have resulted in low detection limits (9 ppt) and excellent recovery (99%) for benzene (EPA 1994c). Screening methods are available for benzene; some may be used at field sites. Immunoassay may be used as a screening and semiquantitative tool (Van Emon and Gerlach 1995).

Methods exist for detection of benzene in other environmental media such as cigarette smoke, gasoline, and jet fuel and its fumes (Brunnemann et al. 1989; Byrd et al. 1990; Ludwig and Eksteen 1988; Poole et al. 1988). HPLC/UV, GC/FID, and GC/MS separation and detection techniques have been used for these analyses. Sensitivity and reliability of these methods cannot be compared because of the lack of data. Few methods have been reported for measurement of benzene in foods; performance data are generally lacking.

7.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of benzene is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of benzene.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect.

Exposure. Methods exist for measuring benzene in breath (Gruenke et al. 1986; Pellizzari et al. 1988; Sherwood and Carter 1970; Wallace et al. 1986), blood (Antoine et al. 1986; Ashley et al. 1992, 1994; Gruenke et al. 1986; Jirka and Bourne 1982; Pekari et al. 1989), and tissues (Bechtold et al. 1988; Rickert et al. 1979). The methods for breath are sensitive and accurate for determining exposure levels of benzene at which health effects have been observed to occur, as well as for background levels in the general population. The methods are relatively precise and selective. Methods for determining benzene in blood are sensitive; based on the limited recovery data available, they appear to be accurate. More information on the performance obtained with different methods would be helpful. The application of GC/MS techniques to the analysis of blood specimens has resulted in a rapid, cost-effective, clinical screening test for common volatile organic compounds, including benzene (DeLeon and Antoine 1985). This test, the VOST (Volatile Organics Screening Test), has demonstrated the presence (down to 0.1 ppb) of a variety of toxic volatile organics in the blood of environmentally sensitive patients and has provided preliminary baseline concentration levels for the test population (DeLeon and Antoine 1985). The data on determination of benzene in urine and tissue samples are very limited. In general, the available methods have limits of detection that are too high to be useful in other than acute exposure situations. Methods that could be used to measure low levels in human tissues would be useful for determining the relationship between chronic low-level exposure and the effects observed in specific tissues.

Methods are available for measuring phenolic benzene metabolites in urine (Bechtold et al. 1991; IARC 1988; Jen and Tsai 1994; Jongeneelen et al. 1987; NIOSH 1974; Ogata and Taguchi 1987). Available methods for determining most benzene metabolites in urine are sufficiently sensitive and reliable to allow measurement of background concentrations in nonoccupationally exposed individuals. However, the phenolic metabolites are not unique to benzene. Improved methods to detect phenolic metabolites are not needed. Sensitive assays have been developed for detection of urinary *trans,trans*-muconic acid

(detection limit 10 μg/L) (Bechtold et al. 1991; Ruppert et al. 1995). Since urinary *trans,trans*-muconic acid concentration can be correlated with benzene exposure, this may provide a useful biomarker of exposure on an individual basis (Bechtold et al. 1991; EPA 1992b; Weaver et al. 2000). In addition, information is needed to assess the effect of co-exposure to other chemicals (e.g., toluene) on urinary muconic acid levels. Also needed are specific biomarkers of cumulative exposure to benzene, based on albumin or hemoglobin adducts, and lymphocyte DNA adducts of N-7-phenylguanine. It would also be useful to develop specific biomarkers of acute- and chronic-duration exposure to benzene based on adducts of muconaldehyde. The levels of such biomarkers formed *in vivo* would be useful later for correlation with toxic effects of acute- or chronic-duration exposure to benzene. S-phenyl-mercapturic acid has also been useful in biological monitoring of benzene exposure in humans and animals. S-phenyl-mercapturic acid can be measured with a sensitivity of about 20 pmol/g globin using HPLC (Hanway et al. 2000). S-phenyl-mercapturic acid levels can also be correlated to environmental benzene exposure (Popp et al. 1994), which may indicate its utility as a biomarker.

Effect. Methods for determining benzene in breath, blood, and tissues and for determining its metabolites in urine could also be used as biomarkers of effect. However, efforts to correlate these measures with observed toxic effects of benzene exposure have been unsuccessful. Other biomarkers of effect (e.g., complete blood cell counts, red and white blood cell counts, chromosomal aberrations, sister chromatid exchanges, and examination of bone marrow) have been suggested for benzene, but they are not specific for benzene exposure. Further development of methods for determining reliable unique biomarkers of effect for benzene would be useful.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods for determining benzene in air (Clark et al. 1984; Gruenke et al. 1986) and water (Brass et al. 1977; EPA 1979, 1984; Hammers and Bosman 1986; Pereira and Hughes 1980), the media of most concern for human exposure, are sensitive enough to measure background levels in the environment and levels at which health effects might occur. Their reliability is limited primarily by the ubiquitous presence of benzene in the environment, which makes contamination a constant problem. The accuracy and precision of some methods for water analyses (e.g., GC/MS) need to be improved to produce more reliable results. Methods for soil and other solid media appear to have the same problems as those for air and water. In addition, there is a lack of information on methods for determining benzene in media such as shellfish, fish, foods, and plants. Although exposure to benzene via ingestion of food is believed to be minimal, standardized methods for these media are needed to better assess the extent of benzene contamination in the environment and the resulting risk of exposure.

7.3.2 Ongoing Studies

The information in Table 7-4 was found as a result of a search of the Federal Research in Progress database (FEDRIP 2005).

The Environmental Health Laboratory Sciences Division of the National Center for Environmental Health, Centers for Disease Control and Prevention, is developing methods for the analysis of benzene and other volatile organic compounds in blood. These methods use purge and trap methodology, high-resolution gas chromatography, and magnetic sector mass spectrometry, which give detection limits in the low parts per trillion (ppt) range.

Table 7-4. Ongoing Studies on Benzene, Analytical Methods[a]

Investigator	Affiliation	Research description	Sponsor
Fales, HM		Analysis of proteins, peptides, and metabolites for carcinogen exposure using mass spectrometry	USDA
Kalman, DA	University of Washington Seattle, Washington	Study of the use of human dosimetry for assessment of exposure to volatile compounds	NIEHS
Kavanaugh, TJ		Study of glutathione biosynthesis as a biomarker of toxic exposure	NSF
O'Brien, RJ		Assessment Environmental Health Breath using PF-GC	NIEHS
Pavel, K	VOC Technologies Inc.; Portland, Oregon	Assessment Environmental Health Breath using PF-GC	NIEHS
Rappaport, SM	University of North Carolina; Chapel Hill, North Carolina	Development and application of biomarkers in the study of exposure to carcinogens	NIEHS
Sabri, MI	Oregon Health Science University; Portland, Oregon	Study of biomarkers of neurotoxicant exposure and neurodegeneration	NIH
Smejtek, PK	VOC Technologies Inc.; Portland, Oregon	A PF-GC for Environmental Health Breath Assessment	NIEHS
Smith, MT	University of California at Berkeley	Study of biomarkers of benzene exposure and genotoxicity	NIEHS
Spencer, PS	Oregon Health Sciences University; Portland, Oregon	Study of neurotoxic chemicals and biomarkers at superfund sites	NIH
Stewart, P		Use of biomarkers to study occupational exposure of carcinogens in order to enhance exposure assessment	Division of cancer epidemiology and genetics
Turtletaub, KW	University of California at Berkeley	Study and development of biomarkers for exposure using accelerator mass spectrometry	NIEHS
Weaver, VM		Use of biomarkers to study benzene exposure in inner city residents	USDA
Wiencke, JK		Use of molecular biomarkers of occupational benzene exposure	USDA

[a]FEDRIP 2005

NIEHS = National Institute of Environmental Health Sciences; NIH = National Institutes of Health; NSF = National Science Foundation; USDA = U.S. Department of Agriculture

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8. REGULATIONS AND ADVISORIES

The international and national regulations and guidelines regarding benzene in air, water, and other media are summarized in Table 8-1.

ATSDR has derived an acute-duration inhalation MRL of 0.009 ppm for benzene based on a LOAEL of 10.2 ppm for immunological effects in mice exposed for 6 hours/day for 6 consecutive days (Rozen et al. 1984). The LOAEL of 10.2 ppm was adjusted from intermittent to continuous exposure ($LOAEL_{ADJ}$=2.55 ppm) and converted to a human equivalent concentration ($LOAEL_{HEC}$=2.55 ppm) using EPA (1994b) methodology for a category 3 gas; an uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans using dosimetric conversion, and 10 to protect sensitive individuals) was applied.

ATSDR has derived an intermediate-duration inhalation MRL of 0.006 ppm for benzene based on a LOAEL of 10 ppm for significantly delayed splenic lymphocyte reaction to foreign antigens evaluated in *in vitro* mixed lymphocyte reaction following the exposure of male C57Bl/6 mice to benzene vapors for 6 hours/day, 5 days/week for 20 exposure days (Rosenthal and Snyder 1987). The concentration was adjusted from intermittent to continuous exposure (LOAELADJ=1.8 ppm) and converted to a human equivalent concentration ($LOAEL_{HEC}$=1.8 ppm) using EPA (1994b) methodology for a category 3 gas; an uncertainty factor of 300 (10 for the use of LOAEL, 3 for extrapolation from animals to humans using dosimetric conversion, and 10 for human variability) was applied.

ATSDR has derived a chronic-duration inhalation MRL of 0.003 ppm for benzene based on the results of benchmark dose (BMD) modeling of B cell counts in workers of shoe manufacturing industries in Tianjin, China (Lan et al. 2004a). The resulting $BMCL_{0.25sd}$ of 0.10 ppm was adjusted from intermittent to continuous exposure ($BMCL_{0.25sdADJ}$=0.03 ppm) using EPA (1994b) methodology; an uncertainty factor of 10 (to protect sensitive individuals) was applied.

ATSDR has derived a chronic-duration oral MRL of 0.0005 mg/kg/day for benzene based on estimation of equivalent chronic-duration oral dosing that would result in effects similar to those observed in the occupationally-exposed workers assessed by Lan et al. (2004a, 2004b). The $BMCL_{0.25sdADJ}$ of 0.03 ppm (0.096 mg/m^3) was converted to an equivalent $BMDL_{0.25sdADJ}$ of 0.014 mg/kg/day for ingested benzene using EPA (1988b) human reference values for inhalation rate (20 m^3/day) and body weight (70 kg) and a factor of 0.5 to adjust for differences in absorption of benzene following inhalation versus oral exposure;

Table 8-1. Regulations and Guidelines Applicable to Benzene

Agency	Description	Information	Reference
INTERNATIONAL			
Guidelines:			
IARC	Carcinogenicity classification	Group 1[a]	IARC 1987, 2004, 2007
WHO	Air quality guidelines	$6x10^{-6}$ unit risk	WHO 2000
	Drinking water quality guidelines	0.01 mg/L[b]	WHO 2004
NATIONAL			
Regulations and Guidelines:			
a. Air			
ACGIH	TLV (TWA)	0.5 ppm[c]	ACGIH 2006
	STEL	2.5 ppm[c]	
EPA	Hazardous air pollutant	Yes	EPA 2004b 42 USC 7412
NAS/NRC	AEGL-1[d]		EPA 2005a
	10 minutes	130 ppm	
	30 minutes	73 ppm	
	60 minutes	52 ppm	
	4 hours	18 ppm	
	8 hours	9.0 ppm	
	AEGL-2[d]		
	10 minutes	2,000 ppm[e]	
	30 minutes	1,100 ppm	
	60 minutes	800 ppm	
	4 hours	400 ppm	
	8 hours	200 ppm	
	AEGL-3[d]		
	10 minutes	9,700 ppm[f]	
	30 minutes	5,600 ppm[e]	
	60 minutes	4,000 ppm[e]	
	4 hours	2,000 ppm[e]	
	8 hours	990 ppm	
NIOSH	REL (10-hour TWA)	0.1 ppm[g]	NIOSH 2005
	STEL	1.0 ppm[g]	
	IDLH	500 ppm[g]	
OSHA	PEL (8-hour TWA) for general industry	1 ppm	OSHA 2005b, 2005e 29 CFR 1910.1000 29 CFR 1910.1028
	PEL (8-hour TWA) for construction industry	1 ppm	OSHA 2005d, 2005f 29 CFR 1926.55, Appendix A 29 CFR 1926.1128

Table 8-1. Regulations and Guidelines Applicable to Benzene

Agency	Description	Information	Reference
NATIONAL *(cont.)*			
OSHA	PEL (8-hour TWA) for shipyard industry	1 ppm	OSHA 2005a,2005b 29 CFR 1915.1000 29 CFR 1915.1028
b. Water			
EPA	Designated as hazardous substances in accordance with Section 311(b)(2)(A) of the Clean Water Act	Yes	EPA 2005b 40 CFR 116.4
	Drinking water standards and health advisories		EPA 2004a
	1-day health advisory for a 10-kg child	0.2 mg/L	
	10-day health advisory for a 10-kg child	0.2 mg/L	
	DWEL	0.1 mg/L	
	10^{-4} Cancer risk	0.1 mg/L	
	National primary drinking water standards		EPA 2002a
	MCLG	Zero	
	MCL	0.005 mg/L	
	Reportable quantities of hazardous substances designated pursuant to Section 311 of the Clean Water Act	10 pounds	EPA 2005c 40 CFR 117.3
	Water quality criteria for human health consumption of:		EPA 2002b
	Water + organism	2.2 µg/L[h]	
	Organism only	51 µg/L[h]	
c. Food			
FDA	Bottled drinking water	0.005 mg/L	FDA 2004 21 CFR 165.110
d. Other			
ACGIH	Carcinogenicity classification	A1[i]	ACGIH 2006
	Biological exposure indices (end of shift)		
	S-phenylmercapturic acid in urine	25 µg/g creatinine	
	t,t-Muconic acid in urine	500 µg/g creatinine	
EPA	Carcinogenicity classification	Group A[j]	IRIS 2007
	Oral slope factor	$1.5x10^{-2}$–$5.5x10^{-2}$ per (mg/kg)/day	
	Inhalation unit risk	$2.2x10^{-6}$–$7.8x10^{-6}$ per µg/m^3	
	RfC	0.03 mg/m^3	
	RfD	$4x10^{-3}$ mg/kg/day	

Table 8-1. Regulations and Guidelines Applicable to Benzene

Agency	Description	Information	Reference
NATIONAL *(cont.)*			
EPA	Superfund, emergency planning, and community right-to-know		
	Designated CERCLA hazardous substance		EPA 2005d 40 CFR 302.4
	Reportable quantity	10 pounds[k]	
	RCRA hazardous waste number	U019	
	Effective date of toxic chemical release reporting	01/01/87	EPA 2005e 40 CFR 372.65
NTP	Carcinogenicity classification	Known human carcinogen	NTP 2005

[a]Group 1: carcinogenic to humans
[b]For substances that are considered to be carcinogenic, the guideline value is the concentration in drinking water associated with an upper-bound excess lifetime cancer risk of 10^{-5} (one additional cancer per 100,000 of the population ingesting drinking water containing the substance at the guideline value for 70 years). Concentrations associated with upper-bound estimated excess lifetime cancer risks of 10^{-4} and 10^{-6} can be calculated by multiplying and dividing, respectively, the guideline value by 10.
[c]Skin notation: refers to the potential significant contribution to the overall exposure by the cutaneous route, including mucous membranes and the eyes, either by contact with vapors or, of probable greater significance, by direct skin contact with the substance.
[d]AEGL-1 is the airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic nonsensory effects. AEGL-2 is the airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape. AEGL-3 is the airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.
[e]Values denoted as having safety considerations against the hazard of explosion, whereas the Lower Explosive Limit (LEL) =14,000 ppm and each value should be ≥10% LEL.
[f]Value denoted as having extreme safety considerations against the hazard of explosion must be taken into account, whereas the LEL =14,000 ppm and each value should be ≥50% LEL.
[g]NIOSH potential occupational carcinogen
[h]This criterion is based on carcinogenicity of 10^{-6} risk.
[i]A1: confirmed human carcinogen
[j]Group A: known human carcinogen
[k]Designated CERCLA hazardous substance pursuant to Section 311(b)(2) and 307(a) of the Clean Water Act, Section 112 of the Clean Air Act, and Section 3001 of RCRA.

ACGIH = American Conference of Governmental Industrial Hygienists; AEGL = Acute Emergency Exposure Guideline Levels; CERCLA = Comprehensive Environmetnal Response, Compensation, and Liability Act; CFR = Code of Federal Regulations; DWEL = drinking water equivalent level; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; IARC = International Agency for Research on Cancer; IDLH = immediately dangerous to life or health; IRIS = Integrated Risk Information System; MCL = maximum contaminant level; MCLG = maximum contaminant level goal; NAS/NRC = National Academy of Sciences/National Research Council; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; PEL = permissible exposure limit; RCRA = Resource Conservation and Recovery Act; REL = recommended exposure limit; RfC = inhalation reference concentration; RfD = oral reference dose; STEL = short-term expsoure limit; TLV = threshold limit values; TWA = time-weighted average; USC = United States Code; WHO = World Health Organization

an uncertainty factor of 30 (10 to protect sensitive individuals and 3 for uncertainty in route-to-route extrapolation) was applied.

EPA (IRIS 2007) derived an inhalation reference concentration (RfC) for benzene of 0.03 mg/m^3 (0.009 ppm) based on the results of BMD modeling of absolute lymphocyte (ALC) data from the occupational epidemiologic study of Rothman et al. (1996a), in which workers were exposed to benzene by inhalation. The resulting BMCL of 7.2 ppm for decreased lymphocyte count was converted to 23.0 mg/m^3 and adjusted from intermittent to continuous exposure ($BMCL_{ADJ}$=8.2 mg/m^3); a total uncertainty factor of 300 (3 for effect-level extrapolation, 10 to protect sensitive individuals, 3 for subchronic-to-chronic extrapolation, and 3 for database deficiencies) was applied.

EPA (IRIS 2007) derived an oral reference dose (RfD) for benzene of 0.004 mg/kg/day, based on the results of BMD modeling of ALC data from the occupational epidemiologic study of Rothman et al. (1996a), in which workers were exposed to benzene by inhalation. The resulting BMCL of 7.2 ppm for decreased lymphocyte count was converted to 23.0 mg/m^3 and adjusted from intermittent to continuous exposure ($BMCL_{ADJ}$=8.2 mg/m^3). Route-to-route extrapolation methodology was applied to convert from inhalation to equivalent oral exposure, resulting in an equivalent oral dose rate of 1.2 mg/kg/day. This value was divided by a total uncertainty factor of 300 (3 for effect-level extrapolation, 10 to protect sensitive individuals, 3 for subchronic-to-chronic extrapolation, and 3 for database deficiencies).

The International Agency for Research on Cancer (IARC) classifies benzene as a Group 1 carcinogen (carcinogenic to humans) (IARC 2004). EPA classified benzene in Category A (known human carcinogen) based on convincing evidence in humans supported by evidence from animal studies. Under EPA's most recent guidelines for carcinogen risk assessment, benzene is characterized as a known human carcinogen for all routes of exposure based on convincing human evidence as well as supporting evidence from animal studies (IRIS 2007). The National Toxicology Programs lists benzene as a "substance known to be carcinogenic," that is, a substance for which the evidence from human studies indicates that there is a causal relationship between exposure to the substance and human cancer (NTP 2005).

The EPA has a current maximum contaminant level (MCL) of 0.005 mg/L for benzene in drinking water (EPA 2002a). The World Health Organization (WHO) has established a guideline value of 0.01 mg/L for benzene in drinking water (WHO 2004).

Benzene is on the list of chemicals in "The Emergency Planning and Community Right-to-Know Act of 1986" (EPA 2005d). Section 313 of Title III of the Superfund Amendments and Reauthorization Act (SARA) requires owners and operators of certain facilities that manufacture, import, process, or otherwise use the chemicals on this list to report annually any release of those chemicals to any environmental media over a specified threshold level (U.S. Congress 1986).

OSHA requires employers of workers who are occupationally exposed to benzene to institute engineering controls and work practices to reduce and maintain employee exposure at or below permissible exposure limits (PEL). If an employer can document that benzene is used in the workplace <30 days/year, the employer can use any combination of engineering controls, work practice controls, or respirators to reduce employee exposure to or below the PEL of 1 ppm (8-hour TWA). Respirators must be provided and used during the time period necessary to install or implement feasible engineering and work practice controls, or where controls are not yet sufficient. Respirators are also required when the employer determines that compliance with the PEL is not feasible with engineering or work practice controls, such as maintenance and repair activities, vessel cleaning, or other operations where exposures are intermittent and limited in duration, and in emergencies (OSHA 2005c).

ACGIH limits exposure to benzene to 0.5 ppm (8-hour TWA) (ACGIH 2006). The National Institute for Occupational Safety and Health (NIOSH 2005) has established a recommended exposure level (REL) of 0.1 ppm (15-minute ceiling limit).

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10. GLOSSARY

Absorption—The taking up of liquids by solids, or of gases by solids or liquids.

Acute Exposure—Exposure to a chemical for a duration of 14 days or less, as specified in the Toxicological Profiles.

Adsorption—The adhesion in an extremely thin layer of molecules (as of gases, solutes, or liquids) to the surfaces of solid bodies or liquids with which they are in contact.

Adsorption Coefficient (K_{oc})—The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

Adsorption Ratio (Kd)—The amount of a chemical adsorbed by sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

Benchmark Dose (BMD)—Usually defined as the lower confidence limit on the dose that produces a specified magnitude of changes in a specified adverse response. For example, a BMD_{10} would be the dose at the 95% lower confidence limit on a 10% response, and the benchmark response (BMR) would be 10%. The BMD is determined by modeling the dose response curve in the region of the dose response relationship where biologically observable data are feasible.

Benchmark Dose Model—A statistical dose-response model applied to either experimental toxicological or epidemiological data to calculate a BMD.

Bioconcentration Factor (BCF)—The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

Biomarkers—Broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility.

Cancer Effect Level (CEL)—The lowest dose of chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or tumors) between the exposed population and its appropriate control.

Carcinogen—A chemical capable of inducing cancer.

Case-Control Study—A type of epidemiological study that examines the relationship between a particular outcome (disease or condition) and a variety of potential causative agents (such as toxic chemicals). In a case-controlled study, a group of people with a specified and well-defined outcome is identified and compared to a similar group of people without outcome.

Case Report—Describes a single individual with a particular disease or exposure. These may suggest some potential topics for scientific research, but are not actual research studies.

Case Series—Describes the experience of a small number of individuals with the same disease or exposure. These may suggest potential topics for scientific research, but are not actual research studies.

Ceiling Value—A concentration of a substance that should not be exceeded, even instantaneously.

Chronic Exposure—Exposure to a chemical for 365 days or more, as specified in the Toxicological Profiles.

Cohort Study—A type of epidemiological study of a specific group or groups of people who have had a common insult (e.g., exposure to an agent suspected of causing disease or a common disease) and are followed forward from exposure to outcome. At least one exposed group is compared to one unexposed group.

Cross-sectional Study—A type of epidemiological study of a group or groups of people that examines the relationship between exposure and outcome to a chemical or to chemicals at one point in time.

Data Needs—Substance-specific informational needs that if met would reduce the uncertainties of human health assessment.

Developmental Toxicity—The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Dose-Response Relationship—The quantitative relationship between the amount of exposure to a toxicant and the incidence of the adverse effects.

Embryotoxicity and Fetotoxicity—Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the insult occurs. The terms, as used here, include malformations and variations, altered growth, and *in utero* death.

Environmental Protection Agency (EPA) Health Advisory—An estimate of acceptable drinking water levels for a chemical substance based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

Epidemiology—Refers to the investigation of factors that determine the frequency and distribution of disease or other health-related conditions within a defined human population during a specified period.

Genotoxicity—A specific adverse effect on the genome of living cells that, upon the duplication of affected cells, can be expressed as a mutagenic, clastogenic, or carcinogenic event because of specific alteration of the molecular structure of the genome.

Half-life—A measure of rate for the time required to eliminate one half of a quantity of a chemical from the body or environmental media.

Immediately Dangerous to Life or Health (IDLH)—The maximum environmental concentration of a contaminant from which one could escape within 30 minutes without any escape-impairing symptoms or irreversible health effects.

Immunologic Toxicity—The occurrence of adverse effects on the immune system that may result from exposure to environmental agents such as chemicals.

Immunological Effects—Functional changes in the immune response.

Incidence—The ratio of individuals in a population who develop a specified condition to the total number of individuals in that population who could have developed that condition in a specified time period.

Intermediate Exposure—Exposure to a chemical for a duration of 15–364 days, as specified in the Toxicological Profiles.

***In Vitro*—**Isolated from the living organism and artificially maintained, as in a test tube.

***In Vivo*—**Occurring within the living organism.

Lethal Concentration$_{(LO)}$ (LC_{LO})—The lowest concentration of a chemical in air that has been reported to have caused death in humans or animals.

Lethal Concentration$_{(50)}$ (LC_{50})—A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose$_{(LO)}$ (LD_{Lo})—The lowest dose of a chemical introduced by a route other than inhalation that has been reported to have caused death in humans or animals.

Lethal Dose$_{(50)}$ (LD_{50})—The dose of a chemical that has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time$_{(50)}$ (LT_{50})—A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL)—The lowest exposure level of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Lymphoreticular Effects—Represent morphological effects involving lymphatic tissues such as the lymph nodes, spleen, and thymus.

Malformations—Permanent structural changes that may adversely affect survival, development, or function.

Minimal Risk Level (MRL)—An estimate of daily human exposure to a hazardous substance that is likely to be without an appreciable risk of adverse noncancer health effects over a specified route and duration of exposure.

Modifying Factor (MF)—A value (greater than zero) that is applied to the derivation of a Minimal Risk Level (MRL) to reflect additional concerns about the database that are not covered by the uncertainty factors. The default value for a MF is 1.

Morbidity—State of being diseased; morbidity rate is the incidence or prevalence of disease in a specific population.

Mortality—Death; mortality rate is a measure of the number of deaths in a population during a specified interval of time.

Mutagen—A substance that causes mutations. A mutation is a change in the DNA sequence of a cell's DNA. Mutations can lead to birth defects, miscarriages, or cancer.

Necropsy—The gross examination of the organs and tissues of a dead body to determine the cause of death or pathological conditions.

Neurotoxicity—The occurrence of adverse effects on the nervous system following exposure to a chemical.

No-Observed-Adverse-Effect Level (NOAEL)—The dose of a chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Effects may be produced at this dose, but they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{ow})—The equilibrium ratio of the concentrations of a chemical in *n*-octanol and water, in dilute solution.

Odds Ratio (OR)—A means of measuring the association between an exposure (such as toxic substances and a disease or condition) that represents the best estimate of relative risk (risk as a ratio of the incidence among subjects exposed to a particular risk factor divided by the incidence among subjects who were not exposed to the risk factor). An OR of greater than 1 is considered to indicate greater risk of disease in the exposed group compared to the unexposed group.

Organophosphate or Organophosphorus Compound—A phosphorus-containing organic compound and especially a pesticide that acts by inhibiting cholinesterase.

Permissible Exposure Limit (PEL)—An Occupational Safety and Health Administration (OSHA) allowable exposure level in workplace air averaged over an 8-hour shift of a 40-hour workweek.

Pesticide—General classification of chemicals specifically developed and produced for use in the control of agricultural and public health pests.

Pharmacokinetics—The dynamic behavior of a material in the body, used to predict the fate (disposition) of an exogenous substance in an organism. Utilizing computational techniques, it provides the means of studying the absorption, distribution, metabolism, and excretion of chemicals by the body.

Pharmacokinetic Model—A set of equations that can be used to describe the time course of a parent chemical or metabolite in an animal system. There are two types of pharmacokinetic models: data-based and physiologically-based. A data-based model divides the animal system into a series of compartments, which, in general, do not represent real, identifiable anatomic regions of the body, whereas the physiologically-based model compartments represent real anatomic regions of the body.

Physiologically Based Pharmacodynamic (PBPD) Model—A type of physiologically based dose-response model that quantitatively describes the relationship between target tissue dose and toxic end points. These models advance the importance of physiologically based models in that they clearly describe the biological effect (response) produced by the system following exposure to an exogenous substance.

Physiologically Based Pharmacokinetic (PBPK) Model—Comprised of a series of compartments representing organs or tissue groups with realistic weights and blood flows. These models require a variety of physiological information: tissue volumes, blood flow rates to tissues, cardiac output, alveolar

ventilation rates, and possibly membrane permeabilities. The models also utilize biochemical information, such as air/blood partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

Prevalence—The number of cases of a disease or condition in a population at one point in time.

Prospective Study—A type of cohort study in which the pertinent observations are made on events occurring after the start of the study. A group is followed over time.

q_1*—The upper-bound estimate of the low-dose slope of the dose-response curve as determined by the multistage procedure. The q_1* can be used to calculate an estimate of carcinogenic potency, the incremental excess cancer risk per unit of exposure (usually μg/L for water, mg/kg/day for food, and μg/m^3 for air).

Recommended Exposure Limit (REL)—A National Institute for Occupational Safety and Health (NIOSH) time-weighted average (TWA) concentration for up to a 10-hour workday during a 40-hour workweek.

Reference Concentration (RfC)—An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer health effects during a lifetime. The inhalation reference concentration is for continuous inhalation exposures and is appropriately expressed in units of mg/m^3 or ppm.

Reference Dose (RfD)—An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure of the human population to a potential hazard that is likely to be without risk of deleterious effects during a lifetime. The RfD is operationally derived from the no-observed-adverse-effect level (NOAEL, from animal and human studies) by a consistent application of uncertainty factors that reflect various types of data used to estimate RfDs and an additional modifying factor, which is based on a professional judgment of the entire database on the chemical. The RfDs are not applicable to nonthreshold effects such as cancer.

Reportable Quantity (RQ)—The quantity of a hazardous substance that is considered reportable under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Reportable quantities are (1) 1 pound or greater or (2) for selected substances, an amount established by regulation either under CERCLA or under Section 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

Reproductive Toxicity—The occurrence of adverse effects on the reproductive system that may result from exposure to a chemical. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

Retrospective Study—A type of cohort study based on a group of persons known to have been exposed at some time in the past. Data are collected from routinely recorded events, up to the time the study is undertaken. Retrospective studies are limited to causal factors that can be ascertained from existing records and/or examining survivors of the cohort.

Risk—The possibility or chance that some adverse effect will result from a given exposure to a chemical.

Risk Factor—An aspect of personal behavior or lifestyle, an environmental exposure, or an inborn or inherited characteristic that is associated with an increased occurrence of disease or other health-related event or condition.

Risk Ratio—The ratio of the risk among persons with specific risk factors compared to the risk among persons without risk factors. A risk ratio greater than 1 indicates greater risk of disease in the exposed group compared to the unexposed group.

Short-Term Exposure Limit (STEL)—The American Conference of Governmental Industrial Hygienists (ACGIH) maximum concentration to which workers can be exposed for up to 15 minutes continually. No more than four excursions are allowed per day, and there must be at least 60 minutes between exposure periods. The daily Threshold Limit Value-Time Weighted Average (TLV-TWA) may not be exceeded.

Standardized Mortality Ratio (SMR)—A ratio of the observed number of deaths and the expected number of deaths in a specific standard population.

Target Organ Toxicity—This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen—A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV)—An American Conference of Governmental Industrial Hygienists (ACGIH) concentration of a substance to which most workers can be exposed without adverse effect. The TLV may be expressed as a Time Weighted Average (TWA), as a Short-Term Exposure Limit (STEL), or as a ceiling limit (CL).

Time-Weighted Average (TWA)—An allowable exposure concentration averaged over a normal 8-hour workday or 40-hour workweek.

Toxic Dose$_{(50)}$ (TD$_{50}$)—A calculated dose of a chemical, introduced by a route other than inhalation, which is expected to cause a specific toxic effect in 50% of a defined experimental animal population.

Toxicokinetic—The absorption, distribution, and elimination of toxic compounds in the living organism.

Uncertainty Factor (UF)—A factor used in operationally deriving the Minimal Risk Level (MRL) or Reference Dose (RfD) or Reference Concentration (RfC) from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using lowest-observed-adverse-effect level (LOAEL) data rather than no-observed-adverse-effect level (NOAEL) data. A default for each individual UF is 10; if complete certainty in data exists, a value of 1 can be used; however, a reduced UF of 3 may be used on a case-by-case basis, 3 being the approximate logarithmic average of 10 and 1.

Xenobiotic—Any chemical that is foreign to the biological system.

APPENDIX A. ATSDR MINIMAL RISK LEVELS AND WORKSHEETS

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) [42 U.S.C. 9601 et seq.], as amended by the Superfund Amendments and Reauthorization Act (SARA) [Pub. L. 99–499], requires that the Agency for Toxic Substances and Disease Registry (ATSDR) develop jointly with the U.S. Environmental Protection Agency (EPA), in order of priority, a list of hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL); prepare toxicological profiles for each substance included on the priority list of hazardous substances; and assure the initiation of a research program to fill identified data needs associated with the substances.

The toxicological profiles include an examination, summary, and interpretation of available toxicological information and epidemiologic evaluations of a hazardous substance. During the development of toxicological profiles, Minimal Risk Levels (MRLs) are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. MRLs are based on noncancer health effects only and are not based on a consideration of cancer effects. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the no-observed-adverse-effect level/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (365 days and longer) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive chemical-induced end point considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that

are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals.

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology and Environmental Medicine, expert panel peer reviews, and agency-wide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published levels. For additional information regarding MRLs, please contact the Division of Toxicology and Environmental Medicine, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop F-32, Atlanta, Georgia 30333.

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Benzene
CAS Numbers: 71-43-2
Date: August 2007
Profile Status: Post Public, Final Draft
Route: [x] Inhalation [ ] Oral
Duration: [x] Acute [ ] Intermediate [ ] Chronic
Graph Key: 46
Species: Mouse

Minimal Risk Level: 0.009 [ ] mg/kg/day [x] ppm

Reference: Rozen MG, Snyder CA, Albert RE. 1984. Depression in B- and T-lymphocyte mitogen-induced blastogenesis in mice exposed to low concentrations of benzene. Toxicol Lett 20:343-349.

Experimental design: Male C57BL/6J mice (7–8/group) were exposed to benzene (0, 10.2, 31, 100, or 301 ppm) in whole-body dynamic inhalation chambers for 6 hours/day for 6 consecutive days. Control mice were exposed to filtered, conditioned air only. Erythrocyte counts were depressed in C57BL/6 mice only at 100 and 301 ppm. The 10.2 ppm exposure level resulted in significant depression of femoral lipopolysaccharide-induced B-colony-forming ability in the absence of a significant depression of total numbers of B cells. At 31 ppm, splenic phytohemagglutinin-induced blastogenesis was significantly depressed without a concomitant significant depression in numbers of T-lymphocytes. Peripheral lymphocyte counts were depressed at all exposure levels. These results demonstrate that short-term inhaled benzene even at low exposure concentrations can alter certain immune associated processes.

Effect noted in study and corresponding doses:

10.2 ppm = No adverse effect on erythrocytes, depressed peripheral lymphocytes and mitogen-induced blastogenesis of femoral B-lymphocytes (less serious LOAEL).

31 ppm = No adverse effect on erythrocytes, depression of mitogen-induced blastogenesis of splenic T-cells.

100 ppm = Depressed erythrocyte counts.

Dose and end point used for MRL derivation:

[ ] NOAEL [x] LOAEL

Uncertainty Factors used in MRL derivation: 300

[ ] 1 [ ] 3 [x] 10 (for use of a LOAEL)
[ ] 1 [x] 3 [ ] 10 (for extrapolation from animals to humans using dosimetric conversion)
[ ] 1 [ ] 3 [x] 10 (for human variability)

Was a conversion factor used from ppm in food or water to a mg/body weight dose? Not applicable.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: The concentration was adjusted for intermittent exposure by multiplying the LOAEL (10.2 ppm) by 6/24 to correct for less than a full day of exposure. The resulting $LOAEL_{ADJ}$ is 2.55 ppm.

According to current EPA (1994b) methodology for calculating a human equivalent concentration (HEC) for extrarespiratory effects of a category 3 gas (such as benzene):

$$LOAEL_{HEC} = LOAEL_{ADJ} \times ([H_{b/g}]_A/[H_{b/g}]_H)$$

where:

$LOAEL_{HEC}$ = The LOAEL dosimetrically adjusted to a human equivalent concentration

$LOAEL_{ADJ}$ = The LOAEL adjusted from intermittent to continuous exposure

$[H_{b/g}]_A/[H_{b/g}]_H$ = The ratio of the blood:gas partition coefficient of the chemical for the laboratory animal species to the human value

If the animal blood:gas partition coefficient is greater than the human blood:gas partition coefficient, a default value of 1 is used for the ratio. According to Wiester et al. (2002), benzene blood:gas partition coefficients for mice and humans are 17.44 and 8.12, respectively. Therefore the default value of 1 is applied, in which case, the $LOAEL_{HEC}$ is equivalent to the $LOAEL_{ADJ}$ = 2.55 ppm.

Was a conversion used from intermittent to continuous exposure? The concentration was adjusted for intermittent exposure by multiplying the LOAEL (10.2 ppm) by 6/24 to correct for less than a full day of exposure. The resulting $LOAEL_{ADJ}$ is 2.55 ppm.

Other additional studies or pertinent information that lend support to this MRL: Increased number of micronucleated polychromatic erythrocytes (MN-PCEs), decreased numbers of granulopoietic stem cells (Toft et al. 1982), lymphopenia (Cronkite et al. 1985), lymphocyte depression, and increased susceptibility to bacterial infection (Rosenthal and Snyder 1985) are among the adverse hematological and immunological effects observed in several other acute-duration inhalation studies. The study by Rozen et al. (1984) shows benzene immunotoxicity (reduced mitogen-induced lymphocyte proliferation) at a slightly lower exposure level than these other studies. C57BI/6J mice were exposed to 0, 10.2, 31, 100, and 301 ppm benzene for 6 days at 6 hours/day. Lymphocyte counts were depressed at all exposure levels while erythrocyte counts were elevated at 10.2 ppm, equal to controls at 31 ppm, and depressed at 100 and 301 ppm. Femoral B-lymphocyte and splenic B-lymphocyte numbers were reduced at 100 ppm. Levels of circulating lymphocytes and mitogen-induced blastogenesis of femoral B-lymphocytes were depressed after exposure to 10.2 ppm benzene for 6 days. Mitogen-induced blastogeneses of splenic T-lymphocytes were depressed after exposure to 31 ppm of benzene for 6 days. In another study, mice exhibited a 50% decrease in the population of erythroid progenitor cells (CFU-E) after exposure to 10 ppm benzene for 5 days, 6 hours/day (Dempster and Snyder 1991). In a study by Wells and Nerland (1991), groups of 4–5 male Swiss-Webster mice were exposed to 0, 3, 25, 55, 105, 199, 303, 527, 1,150, or 2,290 ppm benzene for 6 hours/day for 5 days. The number of leukocytes in peripheral blood and spleen weights were significantly decreased compared with untreated controls at all concentrations ≥25 ppm. Therefore, 3 ppm was the NOAEL and 25 ppm was the LOAEL for these effects. Other end points were not monitored in this study. These data support the choice of Rozen et al. (1984) as a critical study.

Agency Contacts (Chemical Managers): Sharon Wilbur, M.A., Sam Keith, M.S., C.H.P., Obaid Faroon, Ph.D.

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Benzene
CAS Numbers: 71-43-2
Date: August 2007
Profile Status: Post Public, Final Draft
Route: [x] Inhalation [ ] Oral
Duration: [ ] Acute [x] Intermediate [ ] Chronic
Graph Key: 126
Species: Mouse

Minimal Risk Level: 0.006 [ ] mg/kg/day [x] ppm

Reference: Rosenthal GJ, Snyder CA. 1987. Inhaled benzene reduces aspects of cell-mediated tumor surveillance in mice. Toxicol Appl Pharmacol 88:35-43.

Experimental design: Male C57Bl/6 mice were exposed to 10, 30, or 100 ppm of benzene by inhalation 6 hours/day, 5 days/week for 20 exposure days. The number of lymphocytes and their functional capacities were evaluated in spleens of exposed mice. Following the 20 days of exposure, functional capacity of splenic lymphocytes was evaluated in two *in vitro* assays: mixed-lymphocyte culture (MLC) and 51Cr-release cytotoxicity assay. Measured mean daily benzene concentrations in the 10, 30, and 100 ppm groups were 11.1 (±1.5) ppm, 29.5 (±4.4) ppm, and 99.7 (±7.0) ppm, respectively. No changes were observed in the relative proportions of splenic leukocytes, in the percentage of T-cell subsets or in the ratio of T-helper and T-suppressor cells, even at the highest exposure level (100 ppm). Therefore, the functional assays could be normalized for particular lymphocyte populations by using equal numbers of splenic cells. MLC is an *in vitro* measure of alloreactivity (capacity to mount an immune response against foreign antigens). The MLC activity of spleen lymphocytes from 10- and 100-ppm mice was delayed on days 2–4 of culture (relative to air-exposed controls), indicating that benzene exposure causes impaired in vitro alloreactivity (data for the 30-ppm mice were not included in the reported results). This delayed alloreactivity was not due to spleen suppressor cells. The lymphocyte cytotoxic function evaluated in the 51Cr-release assay was also altered; splenic lymphocytes from 100-ppm mice had a significantly reduced lysing capacity. The results indicate that inhalation exposure of mice to benzene has an immunodepressive effect on *in vitro* alloreactivity and cytotoxicity of splenic lymphocytes.

Effect noted in study and corresponding doses:

10 ppm = Significantly delayed splenic lymphocyte reaction to foreign antigens evaluated in *in vitro* mixed lymphocyte reaction (less serious LOAEL).

30 ppm = Results not reported.

100 ppm = Significantly delayed splenic lymphocyte reaction to foreign antigens evaluated in *in vitro* mixed lymphocyte reaction.

Dose and end point used for MRL derivation:

[ ] NOAEL [x] LOAEL

Uncertainty Factors used in MRL derivation: 300

[ ] 1 [ ] 3 [x] 10 (for use of a LOAEL)
[ ] 1 [x] 3 [ ] 10 (for extrapolation from animals to humans using dosimetric conversion)
[ ] 1 [ ] 3 [x] 10 (for human variability)

Was a conversion factor used from ppm in food or water to a mg/body weight dose? Not applicable.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: According to current EPA (1994b) methodology for calculating a human equivalent concentration (HEC) for extrarespiratory effects of a category 3 gas (such as benzene):

$LOAEL_{HEC} = LOAEL_{ADJ} \times ([H_{b/g}]_A/[H_{b/g}]_H)$

where:

$LOAEL_{HEC}$ = The LOAEL dosimetrically adjusted to a human equivalent concentration

$LOAEL_{ADJ}$ = The LOAEL adjusted from intermittent to continuous exposure

$[H_{b/g}]_A/[H_{b/g}]_H$ = The ratio of the blood:gas partition coefficient of the chemical for the laboratory animal species to the human value

If the animal blood:gas partition coefficient is greater than the human blood:gas partition coefficient, a default value of 1 is used for the ratio. According to Wiester et al. (2002), benzene blood:gas partition coefficients for mice and humans are 17.44 and 8.12, respectively. Therefore, the default value of 1 is applied, in which case, the $LOAEL_{HEC}$ is equivalent to the $LOAEL_{ADJ}$.

Was a conversion used from intermittent to continuous exposure? The concentration was adjusted for intermittent exposure by multiplying the LOAEL (10 ppm) by 6 hours/24 hours to correct for less than a full day of exposure and 5 days/7 days to correct for less than a full week of exposure. The resulting $LOAEL_{ADJ}$ is 1.8 ppm.

Other additional studies or pertinent information that lend support to this MRL: Exposure of C57BL mice to 10 ppm benzene for 6 hours/day, 5 days/week caused significant depressions in numbers of lymphocytes (ca. 30% lower than controls) as early as exposure day 32; this effect was also noted at the other scheduled periods of testing (exposure days 66 and 178) (Baarson et al. 1984). Splenic red blood cells were significantly reduced (ca. 15% lower than controls) at exposure days 66 and 178. The failure of the erythrons of benzene-exposed mice to support normal red cell mass was illustrated by the significant reduction in peripheral red cell numbers in these animals at 66 and 178 days of benzene exposure. Green et al. (1981a, 1981b) exposed male CD-1 mice to benzene vapors at concentrations of 0 or 9.6 ppm for 6 hours/day, 5 days/week for 50 days and assessed the effects of exposure on cellularity in the spleen, bone marrow, and peripheral blood. Exposure-related effects included a 90% increase in numbers of multipotential hematopoietic stem cells (CFU-S) (Green et al. 1981a), approximately 25% increase in spleen weight and total splenic nucleated cellularity (Green et al. 1981b), and 80% increase in nucleated RBCs (Green et al. 1981b). The results of Baarson et al. (1984) and Green et al. (1981a, 1981b) are limited for purposes of quantitative risk assessment because a single exposure level was employed. However, they support the choice of Rosenthal and Snyder (1987) as the critical study, which serves as the basis for the intermediate-duration inhalation MRL.

Agency Contacts (Chemical Managers): Sharon Wilbur, M.A., Sam Keith, M.S., C.H.P., Obaid Faroon, Ph.D.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Benzene
CAS Numbers: 71-43-2
Date: August 2007
Profile Status: Post Public, Final Draft
Route: [x] Inhalation [ ] Oral
Duration: [ ] Acute [ ] Intermediate [x] Chronic
Graph Key: 161
Species: Human

Minimal Risk Level: 0.003 [ ] mg/kg/day [x] ppm [ ] mg/m^3

Reference: Lan Q, Zhang L, Li G, et al. 2004a. Hematotoxicity in workers exposed to low levels of benzene. Science 306:1774-1776.

Experimental design: A cross-sectional study was performed on 250 workers (approximately two-thirds female) exposed to benzene at two shoe manufacturing facilities in Tianjin, China, and 140 age- and gender-matched workers in clothing manufacturing facilities that did not use benzene. The benzene-exposed workers had been employed for an average of 6.1±2.9 years. Benzene exposure was monitored by individual organic vapor monitors (full shift) 5 or more times during 16 months prior to phlebotomy. Post-shift urine samples were collected from every worker. Urinary benzene concentrations were highly correlated with mean individual air levels. Benzene was not found (detection limit 0.04 ppm) in workplace and home air samples of control workers taken at three different time periods. Study subjects were categorized into four groups (140 controls, 109 at <1 ppm, 110 at 1–<10 ppm, and 31 at ≥10 ppm) according to mean benzene exposure levels measured twice during the month prior to phlebotomy. Of the 250 exposed workers, 109 were exposed to <1 ppm benzene. Each of these individuals worked at the larger of the two facilities included in the study. Exposure concentrations were generally higher at the smaller facility due to a less adequate ventilation system. Complete blood count (CBC) and differential were analyzed mechanically. Coefficients of variation for all cell counts were <10%.

Mean 1-month benzene exposure levels in the four groups (controls, <1 ppm, 1–<10 ppm, and ≥10 ppm) were <0.04, 0.57±0.24, 2.85±2.11, and 28.73±20.74 ppm, respectively (see Table A-1). An evaluation of potential confounding factors showed that age, gender, cigarette smoking, alcohol consumption, recent infection, and body mass index were associated with at least one hematological end point. The values in Table A-1 represent values that were adjusted to account for these variables. All types of white blood cells (WBCs) and platelets were significantly decreased in the lowest exposure group (<1 ppm), ranging in magnitude from approximately 8 to 15% lower than controls. Although similar statistical analyses for the mid- and high-exposure groups were not included in the study report, decreases in all types of WBCs and platelets were noted at these exposure levels as well; the decreases in the highest exposure group ranged in magnitude from 15 to 36%. Lymphocyte subset analysis revealed significantly decreased CD4+-T cells, CD4+/CD8+ ratio, and B cells. Hemoglobin concentrations were significantly decreased only within the highest (≥10 ppm) exposure group. Tests for a linear trend using benzene air level as a continuous variable were significant for platelets and all WBC measures except monocytes and CD8+-T cells. Upon restricting the linear trend analyses to workers exposed to <10 ppm benzene, excluding controls, inverse associations remained for total WBCs, granulocytes, lymphocytes, B cells, and platelets. In order to evaluate the effect of past benzene exposures on the hematological effects observed in this study, the authors compared findings for a group of workers who had been exposed to <1 ppm benzene over the previous year (n=60) and a subset who also had <40 ppm-years lifetime cumulative benzene exposure (n=50). The authors stated that the same cell types were significantly reduced in these groups, but did not provide further information of the magnitude (i.e., percent change) of

the hematological effects observed. These data suggest that the 1-month benzene exposure results could be used as an indicator of longer term low-level benzene hematotoxicity. To demonstrate that the observed effects were attributable to benzene, significantly decreased levels of WBCs, granulocytes, lymphocytes, and B cells were noted in a subgroup (n=30; mean 1-month exposure level of 0.29±0.15 ppm) of the <1 ppm group for which exposure to other solvents was negligible.

Table A-1. Significantly Reduced Blood Values in Workers Exposed to Benzene in Tianjin, China (Adapted from Lan et al. 2004a)

	Mean exposure level in ppm[a] (number of subjects)			
End point	<0.04 (140)	0.57±0.24 (109)	2.85±2.11 (110)	28.73±20.74 (31)
WBCs[b]	6,480±1,710	5,540±1,220[d]	5,660±1,500	4,770±892
Granulocytes[b]	4,110±1,410	3,360±948[d]	3,480±1,170	2,790±750
Monocytes[b]	241±92	217±97[d]	224±93	179±74
Lymphocytes[b]	2,130±577	1,960±541[d]	1,960±533	1,800±392
CD4+-T cells[b]	742±262	635±187[d]	623±177	576±188
CD4+/CD8+ ratio	1.46±0.58	1.26±0.41[d]	1.22±0.45	1.09±0.35
B cells[b]	218±94	186±95[d]	170±75	140±101
Platelets[c]	230±59.7	214±48.8[d]	200±53.4	172±44.8

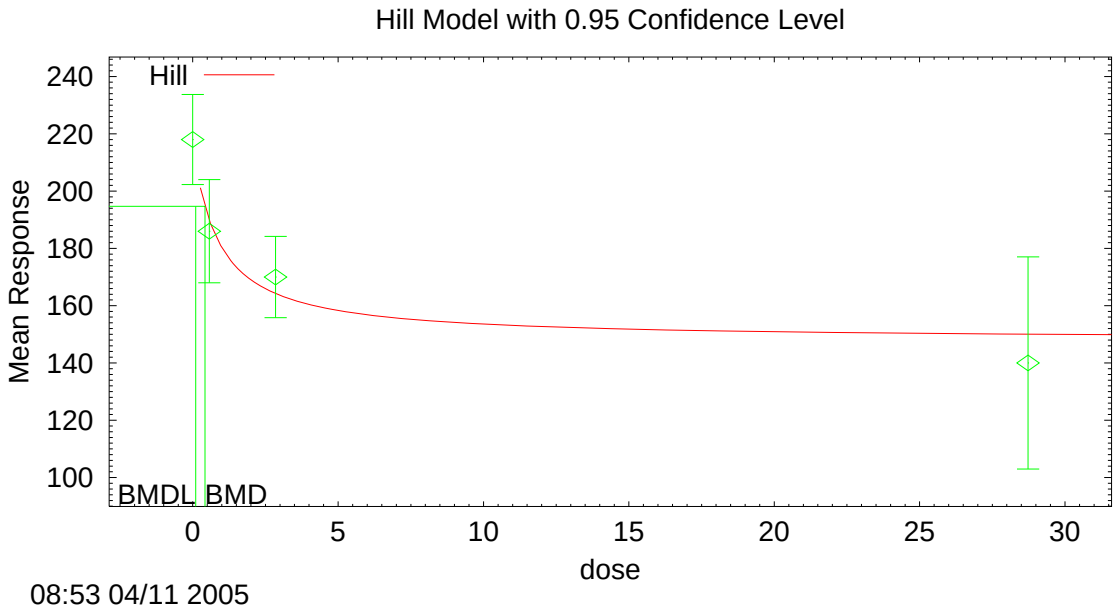
[a]Arithmetic mean of an average of two measurements per subject collected during the month prior to phlebotomy
[b]Mean cell numbers per microliter blood±standard deviation
[c]Mean number of platelets ($x10^3$)
[d]Statistically significantly lower than controls (p<0.05) by linear regression on ln of each end point

Effect noted in study and corresponding doses: As shown in Table A-1, exposure-response relationships were noted for several blood factors. Benzene-induced decreased B cell count was selected as the critical effect for benchmark dose (BMD) modeling because it represented the highest magnitude of effect (i.e., B cell count in the highest exposure group was approximately 36% lower than that of controls). A BMD modeling approach was selected to identify the point of departure because the critical study (Lan et al. 2004a) identified a LOAEL in the absence of a NOAEL.

Dose and end point used for MRL derivation: 0.10 ppm ($BMCL_{0.25sd}$) for decreased B cell count.

All continuous variable models in the EPA Benchmark Dose Software (Version 1.3.2) were fit to the B cell count data shown in Table A-1. Visual inspection of the plots of observed versus expected values for B cell counts indicated that the Hill model provided the only adequate fit of the data set (see Figure A-1). A benchmark response (BMR) of 0.25 sd below the control mean B cell count was selected because it resulted in a $BMC_{0.25sd}$ of 0.42 ppm and its lower 95% confidence limit ($BMCL_{0.25sd}$) of 0.10 ppm (Figure A-1), which are below the mean exposure level of the lowest exposure group (0.57 ppm) for which a statistically significant decrease in mean B cell count (186 versus 218 in controls, see Table A-1) was observed. Although Lan et al. (2004a, 2004b) noted significantly decreased levels of WBCs, granulocytes, lymphocytes, and B cells in a subgroup (n=30; mean 1-month exposure level of 0.29±0.15 ppm) of the 0.57 ppm exposure group, this subgroup could not be included in the BMD analysis because the study authors did not include the means and standard deviations for the decreased blood factors, nor did they provide quantitative information regarding the remaining 70 subjects in the 0.57 ppm exposure group (n=109). Assuming that the 0.29 ppm exposure level may represent a minimally adverse exposure level, it seems reasonable to accept the $BMCL_{0.25sd}$ of 0.10 ppm as the point of departure for deriving a chronic-duration inhalation MRL for benzene.

Figure A-1. Observed and Predicted B Cell Counts in Human Subjects Occupationally Exposed to Benzene. BMD=$BMC_{0.25sd}$=0.42 ppm; BMDL=$BMCL_{0.25sd}$=0.10 ppm



The computer output for fitting of the Hill model to B cell counts in human subjects occupationally exposed to benzene (Lan et al. 2004a) follows.

```
=====================================================================
        Hill Model. $Revision: 2.1 $ $Date: 2000/10/11 21:21:23 $
        Input Data File: C:\ATSDR\BENZENE\BMD FILES\BENZENELANBCELLS.(d)
        Gnuplot Plotting File:  C:\ATSDR\BENZENE\BMD
FILES\BENZENELANBCELLS.plt
                                          Mon Nov 20 09:27:13 2006
 ====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)

   Dependent variable = MEAN
   Independent variable = ppm
   rho is set to 0
   Power parameter restricted to be greater than 1
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
```

Default Initial Parameter Values

alpha =	8224.64	
rho =	0	Specified
intercept =	218	
v =	-78	
n =	0.572459	
k =	1.5675	

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -n have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	alpha	rho	intercept	v	k
alpha	1	0	0	0	0
rho	0	1	0	0	0
intercept	0	0	1	0	0
v	0	0	0	1	0
k	0	0	0	0	1

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	8027.09	1
rho	0	1
intercept	217.113	1
v	-69.0144	1
n	1	NA
k	0.878186	1

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	140	218	94	217	89.6	0.0099
0.57	109	186	95	190	89.6	-0.0441
2.85	110	170	75	164	89.6	0.063
28.73	31	140	101	150	89.6	-0.113

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)

```
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

 Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


Degrees of freedom for Test A1 vs fitted <= 0

                       Likelihoods of Interest

            Model      Log(likelihood)   DF        AIC
             A1        -1947.632025       5    3905.264050
             A2        -1943.411648       8    3902.823297
           fitted      -1948.162584       4    3904.325168
              R        -1962.157799       2    3928.315597

 Test 1:  Does response and/or variances differ among dose levels
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous (A1 vs A2)
 Test 3:  Does the Model for the Mean Fit (A1 vs. fitted)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df     p-value

   Test 1              37.4923          6          <.0001
   Test 2              8.44075          3         0.03773
   Test 3              1.06112          0              NA

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels.
It seems appropriate to model the data

The p-value for Test 2 is less than .05.  Consider running a
non-homogeneous variance model

NA - Degrees of freedom for Test 3 are less than or equal to 0.  The Chi-
Square test for fit is not valid

Benchmark Dose Computation
Specified effect =          0.25

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMC =       0.42196

            BMCL =      0.104163
```

Although Test 3 (mean fit) produced an invalid Chi-Square test (degrees of freedom ≤0), visual inspection of the observed vs expected B cell counts from the Hill model output (Figure A-1) resulted in the determination that the predicted B cell counts adequately reflect the observed values and that the

associated $BMCL_{0.25sd}$ of 0.104163 provides an appropriate point of departure for deriving a chronic-duration inhalation MRL for benzene.

[ ] NOAEL [ ] LOAEL

Uncertainty Factors used in MRL derivation: 10

[ ] 1 [ ] 3 [ ] 10 (for use of a LOAEL)
[ ] 1 [ ] 3 [ ] 10 (for extrapolation from animals to humans)
[ ] 1 [ ] 3 [x] 10 (for human variability)

Was a conversion factor used from ppm in food or water to a mg/body weight dose? Not applicable.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? The $BMCL_{0.25sd}$ of 0.10 ppm was adjusted from the 8-hour TWA to a continuous exposure concentration ($BMCL_{0.25sdADJ}$) as follows:

$BMCL_{0.25sdADJ}$ = $BMCL_{0.25sd}$ x (8 hours/24 hours) x (6 days/7 days)

Therefore:

$BMCL_{0.25sdADJ}$ = 0.10 ppm x (8 hours/24 hours) x (6 days/7 days)

$BMCL_{0.25sdADJ}$ = 0.03 ppm

Other additional studies or pertinent information that lend support to this MRL: Lan et al. (2004a, 2004b) was selected as the critical study for derivation of a chronic-duration inhalation MRL because it (1) was well designed, (2) provided adequate exposure-response information, (3) employed individual exposure monitoring data collected for up to 16 months prior to blood testing, (4) demonstrated effects that did not appear to be significantly influenced by previous high-level exposures, and (5) included larger numbers of subjects than previous studies (Qu et al. 2002, 2003; Rothman et al. 1996a, 1996b; Ward et al. 1996). In addition, Lan et al. (2004a, 2004b) measured lymphocyte subsets and colony formation from hematopoietic progenitor cells as measures of toxicity.

Previously conducted epidemiology studies provide support to the findings of Lan et al. (2004a). Qu et al. (2002, 2003) compared hematologic values among 105 healthy workers (51 men, 54 women) in industries with a history of benzene usage (Tianjin, China) and 26 age- and gender-matched workers in industries that did not use benzene. Benzene-exposed workers were chosen based on at least 3 years of exposure history. The mean duration of occupational exposure to benzene was 9.7 years (SD=6.2 years). At the time of the study, benzene exposure was monitored by individual organic vapor monitors at 1-week intervals for 4 weeks prior to collection of blood samples for analysis. Measured benzene levels were averaged for each individual to produce a 4-week mean exposure level. Exposure-response relationships were assessed according to ranges of benzene levels (unexposed, >0–5, >5–15, >15–30, and >30 ppm). Benzene hematotoxicity was assessed by mechanical counts of total WBCs, red blood cells (RBCs), and platelets. The WBC differential was hand-counted on a total of 900 cells. Calculations of the numbers of various WBC types were based on total WBCs and differential counts. The mean 4-week benzene level in the control group was 0.004±0.003 ppm. Among all the benzene-exposed workers, the mean 4-week benzene exposure level was 5.2±7.3 ppm. Within the >0–5, >5–15, >15–30, and >30 ppm exposure categories, mean 4-week benzene levels were 2.26±1.35, 8.67±2.44, 19.9±3.1, and

51.8±43.3 ppm, respectively. A significant exposure-related reduction in the numbers of neutrophils (ranging in magnitude from 12% in the 2.26 ppm exposure group to 31% in the 51.8 ppm exposure group) was observed in all four groups of benzene-exposed workers, relative to controls. Significantly reduced numbers of RBCs (approximately 11–16% lower than controls) were also noted in all benzene-exposed groups. Significantly reduced total WBCs were seen in the highest (>30 ppm) exposure group. The study authors identified a subgroup (within the >0–5 ppm exposure group) of 16 women with no measured exposure levels exceeding 0.5 ppm (4-week mean benzene exposure level of 0.14±0.04 ppm) and reported significantly reduced total WBCs, neutrophils, and RBCs in this subgroup as well. However, these results are based on a small number of workers within the larger group and the reduced counts of total WBCs, neutrophils, and RBCs within this subgroup are –much greater in magnitude than those reported for the main (>0–5 ppm) exposure group, rendering the results in this subgroup of questionable value for purposes of risk assessment. Qu et al. (2002, 2003) clearly identified a LOAEL of 2.26 ppm for significantly reduced total WBCs, neutrophils, and RBCs, and provided indication of benzene-induced changes in some hematological values at exposure levels lower than the current industry 8-hour TWA of 1 ppm.

Rothman et al. (1996a, 1996b) performed a cross-sectional study in 1992 on 44 healthy workers (23 males, 21 females) in Chinese (Shanghai) industries with a history of benzene usage and 44 age- and gender-matched workers in industries that did not use benzene. The mean duration of occupational exposure to benzene was 6.3 years (SD=4.4 years). At the time of the study, benzene exposure was monitored by individual organic passive dosimetry badges on 5 separate days during 1 to 2 weeks prior to the collection of blood and urine samples for analysis. Benzene hematotoxicity was assessed by mechanical counts of total WBCs, absolute lymphocytes (ALC), RBCs, and platelets, as well as hemoglobin value and mean corpuscular volume (MCV). The WBC differential was also hand-counted on 100 cells. Abnormal counts were reviewed by hand. Mean (geometric mean of the five exposure samples) 8-hour TWAs for the benzene-exposed workers ranged from 1 to 238 ppm (median 8-hour TWA of 31 ppm). Benzene-exposed workers exhibited statistically significantly reduced numbers of total WBCs, ALC, RBCs, and platelets (approximately 12, 21, 6, and 23% lower, respectively) and significantly increased MCV (approximately 3% higher), relative to unexposed workers. The results were comparable in both men and women. Among the benzene-exposed workers whose mean exposure levels were >31 ppm (median 8-hour TWA of 91.9 ppm; n=22), all measured blood parameters were significantly different from controls; only ALC, RBCs, and platelets were significantly lower in benzene workers with mean exposures of <31 ppm (median 8-hour TWA of 13.6; n=22), compared with controls. In a subgroup of benzene-exposed workers whose measured benzene exposure levels did not exceed 31 ppm on any of the five sampling days (median 8–hour TWA of 7.6 ppm; n=11), significantly reduced ALC (approximately 16% lower than controls) was noted.

In a nested case-control study of a cohort of workers in the Pliofilm production departments of a rubber products manufacturer in Ohio (Ward et al. 1996), incident cases were defined as the first occurrence of a low WBC or RBC count, and matched controls were chosen from those tested within approximately 6 months of the case's blood test date. Hematologic screening data were available for 657 of 1,037 individuals employed at the plant from 1939 through 1976. A total of 21,710 blood test records were identified; the number of blood tests per individual ranged from 1 to 354, but the majority of subjects had five or fewer blood tests. All blood tests were taken from 1940 through 1975, the majority of which were routine hematological screening tests. Benzene exposures were estimated using a job exposure matrix developed by Rinsky et al. (1987). The effects of benzene exposure in the 30, 90, and 180 days prior to the blood test date, as well as cumulative exposure up until the blood test date, were examined using conditional logistic regression. A total of 78 cases and 5,637 controls were included in the WBC analysis and 105 cases and 8,489 controls in the RBC analysis, all of whom had worked only within the rubber hydrochloride departments during the 180 days prior to the selected blood sample date. The maximum daily benzene exposure estimate was 34 ppm. A strong exposure-response relationship

was noted for WBCs, and all of the exposure metrics selected showed a significant relationship with low blood count. A weak positive exposure-response relationship was observed for RBCs, which was significant for the dose metric of cumulative exposure up until the blood test date. The study authors noted that there was no evidence for a threshold for hematologic effects and suggested that exposure to benzene levels <5 ppm may result in hematologic suppression.

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MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Benzene
CAS Numbers: 71-43-2
Date: August 2007
Profile Status: Post Public, Final Draft
Route: [ ] Inhalation [x] Oral
Duration: [ ] Acute [ ] Intermediate [x] Chronic
Graph Key: 45
Species: Human

Minimal Risk Level: 0.0005 [x] mg/kg/day [ ] ppm

Reference: Lan Q, Zhang L, Li G, et al. 2004a. Hematotoxicity in workers exposed to low levels of benzene. Science 306:1774-1776.

Experimental design: The chronic-duration oral MRL for benzene is based on route-to-route extrapolation of the results of benchmark dose analysis of a hematological endpoint (B cell count) assessed in 250 workers (approximately two-thirds female) exposed to benzene at two shoe manufacturing facilities in Tianjin, China, and 140 age- and gender-matched workers in clothing manufacturing facilities that did not use benzene. See the MRL worksheet for the chronic-duration inhalation MRL for details of study design.

Effect noted in study and corresponding doses: As described in the MRL worksheet for the chronic-duration inhalation MRL (see also Table A-1), exposure-response relationships were noted for several blood factors. Benzene-induced decreased B cell count was selected as the critical effect for benchmark dose (BMD) modeling because it represented the highest magnitude of effect (i.e., B cell count in the highest exposure group was approximately 36% lower than that of controls). A BMD modeling approach was selected to identify the point of departure because the critical study (Lan et al. 2004a) identified a LOAEL in the absence of a NOAEL.

Dose and end point used for MRL derivation: $BMCL_{0.25sdADJ}$ of 0.014 mg/kg/day for decreased B cell count, resulting from route-to-route extrapolation of the $BMCL_{0.25sdADJ}$ of 0.03 ppm described in the MRL worksheet for the chronic-duration inhalation MRL.

Results of toxicokinetic studies of inhaled benzene in humans (Nomiyama and Nomiyama 1974a; Pekari et al. 1992; Srbova et al. 1950) and inhaled and orally-administered benzene in rats and mice (Sabourin et al. 1987) indicate that absorption of benzene at relatively low levels of exposure is approximately 50% of an inhaled dose and essentially 100% of an oral dose. Based on these assumptions, inhalation data can be used to estimate equivalent oral doses that would be expected to similarly affect the critical targets of benzene toxicity. Therefore, the point of departure for the chronic-duration inhalation MRL for benzene, namely the $BMCL_{0.25sdADJ}$ of 0.03 ppm for decreased B cell counts in benzene-exposed workers (Lan et al. 2004a, 2004b), serves as the point of departure for deriving the chronic-duration oral MRL as well.

The point of departure (in ppm) was converted to mg/m^3 using the molecular weight of 78.11 for benzene and assuming 25 °C and 760 mm Hg:

$BMCL_{0.25sdADJ}$ of 0.03 ppm x 78.11/24.45 = 0.096 mg/m^3

The $BMCL_{0.25sdADJ}$ of 0.096 mg/m^3 for inhaled benzene was converted to an equivalent $BMDL_{0.25sdADJ}$ for ingested benzene using EPA (1988b) human reference values for inhalation rate (20 m^3/day) and body

weight (70 kg) and a factor of 0.5 to adjust for differences in absorption of benzene following inhalation versus oral exposure (50 versus 100%, respectively) as follows:

$BMDL_{0.25sdADJ}$ = $BMCL_{0.25sdADJ}$ of 0.096 mg/m^3 x 20 m^3/day x 0.5 ÷ 70 kg = 0.014 mg/kg/day

[ ] NOAEL [ ] LOAEL

Uncertainty Factors used in MRL derivation: 30

[ ] 1 [ ] 3 [ ] 10 (for use of a LOAEL)
[ ] 1 [ ] 3 [ ] 10 (for extrapolation from animals to humans)
[ ] 1 [ ] 3 [x] 10 (for human variability)
[ ] 1 [x] 3 [ ] 10 (for uncertainty in route-to-route extrapolation)

Was a conversion factor used from ppm in food or water to a mg/body weight dose? Not applicable.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? The $BMCL_{0.25sd}$ of 0.10 ppm was adjusted from the 8-hour TWA to a continuous exposure concentration ($BMCL_{0.25sdADJ}$) as follows:

$BMCL_{0.25sdADJ}$ = $BMCL_{0.25sd}$ x (8 hours/24 hours) x (6 days/7 days)

Therefore:

$BMCL_{0.25sdADJ}$ = 0.10 ppm x (8 hours/24 hours) x (6 days/7 days)

$BMCL_{0.25sdADJ}$ = 0.03 ppm

Other additional studies or pertinent information that lend support to this MRL: Results of toxicokinetic studies of inhaled benzene in humans (Nomiyama and Nomiyama 1974a; Pekari et al. 1992; Srbova et al. 1950) and inhaled and orally-administered benzene in rats and mice (Sabourin et al. 1987) indicate that absorption of benzene at relatively low levels of exposure is approximately 50% of an inhaled dose and essentially 100% of an oral dose. Based on these assumptions, inhalation data can be used to estimate equivalent oral doses that would be expected to similarly affect the critical targets of benzene toxicity. See the chronic-duration inhalation MRL worksheet for additional information that supports the selection of the principal study and critical effect for deriving the chronic-duration inhalation MRL.

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APPENDIX B. USER'S GUIDE

Chapter 1

Public Health Statement

This chapter of the profile is a health effects summary written in non-technical language. Its intended audience is the general public, especially people living in the vicinity of a hazardous waste site or chemical release. If the Public Health Statement were removed from the rest of the document, it would still communicate to the lay public essential information about the chemical.

The major headings in the Public Health Statement are useful to find specific topics of concern. The topics are written in a question and answer format. The answer to each question includes a sentence that will direct the reader to chapters in the profile that will provide more information on the given topic.

Chapter 2

Relevance to Public Health

This chapter provides a health effects summary based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information. This summary is designed to present interpretive, weight-of-evidence discussions for human health end points by addressing the following questions:

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

The chapter covers end points in the same order that they appear within the Discussion of Health Effects by Route of Exposure section, by route (inhalation, oral, and dermal) and within route by effect. Human data are presented first, then animal data. Both are organized by duration (acute, intermediate, chronic). *In vitro* data and data from parenteral routes (intramuscular, intravenous, subcutaneous, etc.) are also considered in this chapter.

The carcinogenic potential of the profiled substance is qualitatively evaluated, when appropriate, using existing toxicokinetic, genotoxic, and carcinogenic data. ATSDR does not currently assess cancer potency or perform cancer risk assessments. Minimal Risk Levels (MRLs) for noncancer end points (if derived) and the end points from which they were derived are indicated and discussed.

Limitations to existing scientific literature that prevent a satisfactory evaluation of the relevance to public health are identified in the Chapter 3 Data Needs section.

Interpretation of Minimal Risk Levels

Where sufficient toxicologic information is available, ATSDR has derived MRLs for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action, but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans.

MRLs should help physicians and public health officials determine the safety of a community living near a chemical emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Chapter 2, "Relevance to Public Health," contains basic information known about the substance. Other sections such as Chapter 3 Section 3.9, "Interactions with Other Substances," and Section 3.10, "Populations that are Unusually Susceptible" provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology that the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the most sensitive end point which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen end point are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest no-observed-adverse-effect level (NOAEL) that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor (UF) of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a substance-specific MRL are provided in the footnotes of the levels of significant exposure (LSE) tables.

Chapter 3

Health Effects

Tables and Figures for Levels of Significant Exposure (LSE)

Tables and figures are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species, MRLs to humans for noncancer end points, and EPA's estimated range associated with an upper- bound individual lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. Use the LSE tables and figures for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of NOAELs, LOAELs, or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE Table 3-1 and Figure 3-1 are shown. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

LEGEND

See Sample LSE Table 3-1 (page B-6)

(1) Route of Exposure. One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. Typically when sufficient data exist, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure, i.e., inhalation, oral, and dermal (LSE Tables 3-1, 3-2, and 3-3, respectively). LSE figures are limited to the inhalation (LSE Figure 3-1) and oral (LSE Figure 3-2) routes. Not all substances will have data on each route of exposure and will not, therefore, have all five of the tables and figures.

(2) Exposure Period. Three exposure periods—acute (less than 15 days), intermediate (15–364 days), and chronic (365 days or more)—are presented within each relevant route of exposure. In this example, an inhalation study of intermediate exposure duration is reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Health Effect. The major categories of health effects included in LSE tables and figures are death, systemic, immunological, neurological, developmental, reproductive, and cancer. NOAELs and LOAELs can be reported in the tables and figures for all effects but cancer. Systemic effects are further defined in the "System" column of the LSE table (see key number 18).

(4) Key to Figure. Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 18 has been used to derive a NOAEL and a Less Serious LOAEL (also see the two "18r" data points in sample Figure 3-1).

(5) Species. The test species, whether animal or human, are identified in this column. Chapter 2, "Relevance to Public Health," covers the relevance of animal data to human toxicity and Section 3.4, "Toxicokinetics," contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(6) Exposure Frequency/Duration. The duration of the study and the weekly and daily exposure regimens are provided in this column. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 18), rats were exposed to “Chemical x” via inhalation for 6 hours/day, 5 days/week, for 13 weeks. For a more complete review of the dosing regimen, refer to the appropriate sections of the text or the original reference paper (i.e., Nitschke et al. 1981).

(7) System. This column further defines the systemic effects. These systems include respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, and dermal/ocular. "Other" refers to any systemic effect (e.g., a decrease in body weight) not covered in these systems. In the example of key number 18, one systemic effect (respiratory) was investigated.

(8) NOAEL. A NOAEL is the highest exposure level at which no harmful effects were seen in the organ system studied. Key number 18 reports a NOAEL of 3 ppm for the respiratory system, which was used to derive an intermediate exposure, inhalation MRL of 0.005 ppm (see footnote "b").

(9) LOAEL. A LOAEL is the lowest dose used in the study that caused a harmful health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific end point used to quantify the adverse effect accompanies the LOAEL. The respiratory effect reported in key number 18 (hyperplasia) is a Less Serious LOAEL of 10 ppm. MRLs are not derived from Serious LOAELs.

(10) Reference. The complete reference citation is given in Chapter 9 of the profile.

(11) CEL. A CEL is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases.

(12) Footnotes. Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. Footnote "b" indicates that the NOAEL of 3 ppm in key number 18 was used to derive an MRL of 0.005 ppm.

LEGEND

See Sample Figure 3-1 (page B-7)

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(13) Exposure Period. The same exposure periods appear as in the LSE table. In this example, health effects observed within the acute and intermediate exposure periods are illustrated.

(14) Health Effect. These are the categories of health effects for which reliable quantitative data exists. The same health effects appear in the LSE table.

(15) Levels of Exposure. Concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(16) NOAEL. In this example, the open circle designated 18r identifies a NOAEL critical end point in the rat upon which an intermediate inhalation exposure MRL is based. The key number 18 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 3 ppm (see entry 18 in the table) to the MRL of 0.005 ppm (see footnote "b" in the LSE table).

(17) CEL. Key number 38m is one of three studies for which CELs were derived. The diamond symbol refers to a CEL for the test species-mouse. The number 38 corresponds to the entry in the LSE table.

(18) Estimated Upper-Bound Human Cancer Risk Levels. This is the range associated with the upper-bound for lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. These risk levels are derived from the EPA's Human Health Assessment Group's upper-bound estimates of the slope of the cancer dose response curve at low dose levels (q_1*).

(19) Key to LSE Figure. The Key explains the abbreviations and symbols used in the figure.

SAMPLE

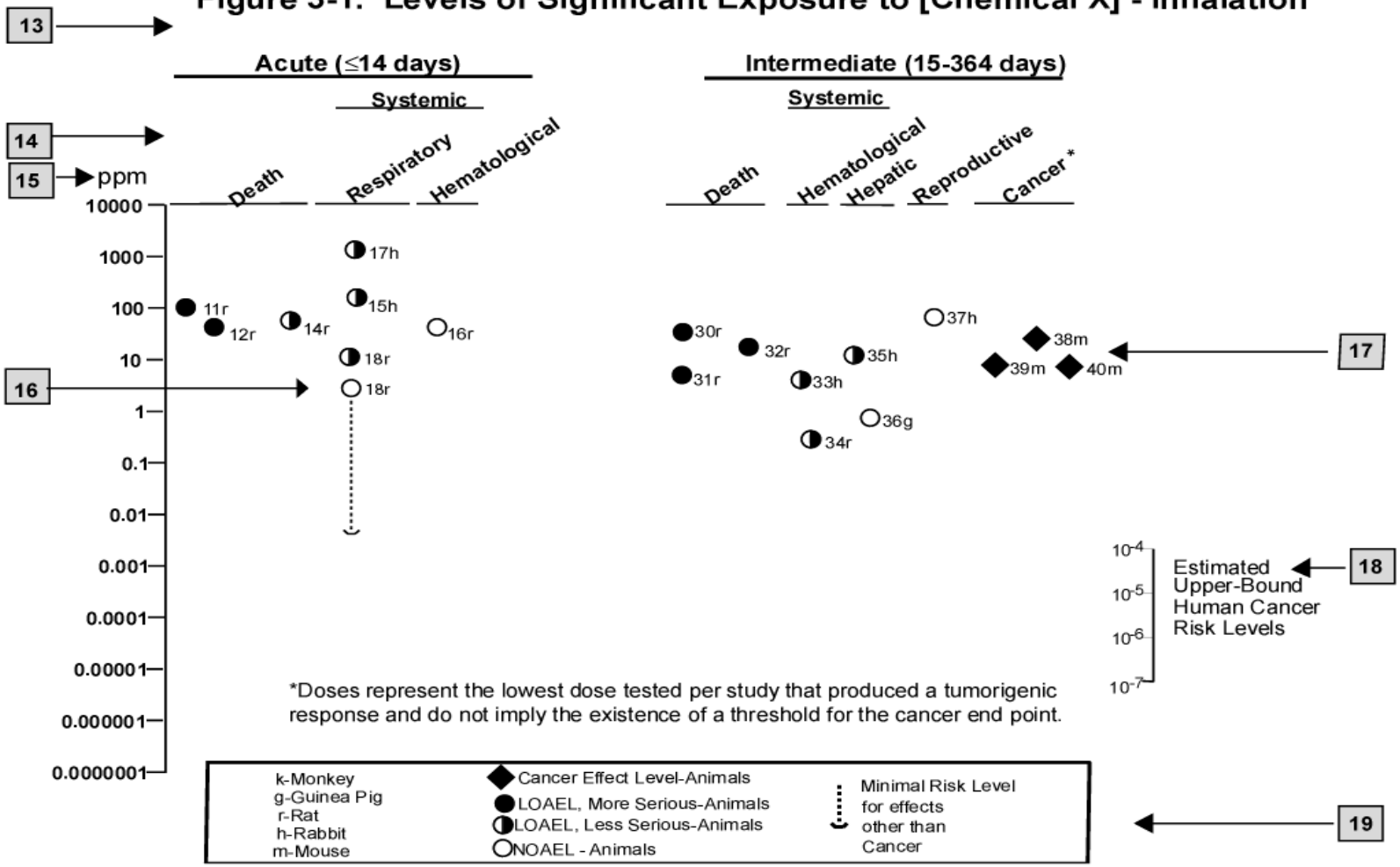
1 → **Table 3-1. Levels of Significant Exposure to [Chemical x] – Inhalation**

	Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
2 →	INTERMEDIATE EXPOSURE							
		5	6	7	8	9		10
3 →	Systemic	↓	↓	↓	↓	↓		↓
4 →	18	Rat	13 wk 5 d/wk 6 hr/d	Resp	3[b]	10 (hyperplasia)		Nitschke et al. 1981
	CHRONIC EXPOSURE							
	Cancer						11	
							↓	
	38	Rat	18 mo 5 d/wk 7 hr/d				20 (CEL, multiple organs)	Wong et al. 1982
	39	Rat	89–104 wk 5 d/wk 6 hr/d				10 (CEL, lung tumors, nasal tumors)	NTP 1982
	40	Mouse	79–103 wk 5 d/wk 6 hr/d				10 (CEL, lung tumors, hemangiosarcomas)	NTP 1982

12 → [a] The number corresponds to entries in Figure 3-1.
[b] Used to derive an intermediate inhalation Minimal Risk Level (MRL) of 5×10^{-3} ppm; dose adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animal to humans, 10 for human variability).

SAMPLE

Figure 3-1. Levels of Significant Exposure to [Chemical X] - Inhalation



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APPENDIX C. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
ACOEM	American College of Occupational and Environmental Medicine
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
AED	atomic emission detection
AFID	alkali flame ionization detector
AFOSH	Air Force Office of Safety and Health
ALT	alanine aminotransferase
AML	acute myeloid leukemia
AOAC	Association of Official Analytical Chemists
AOEC	Association of Occupational and Environmental Clinics
AP	alkaline phosphatase
APHA	American Public Health Association
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AWQC	Ambient Water Quality Criteria
BAT	best available technology
BCF	bioconcentration factor
BEI	Biological Exposure Index
BMD	benchmark dose
BMR	benchmark response
BSC	Board of Scientific Counselors
C	centigrade
CAA	Clean Air Act
CAG	Cancer Assessment Group of the U.S. Environmental Protection Agency
CAS	Chemical Abstract Services
CDC	Centers for Disease Control and Prevention
CEL	cancer effect level
CELDS	Computer-Environmental Legislative Data System
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
Ci	curie
CI	confidence interval
CL	ceiling limit value
CLP	Contract Laboratory Program
cm	centimeter
CML	chronic myeloid leukemia
CPSC	Consumer Products Safety Commission
CWA	Clean Water Act
DHEW	Department of Health, Education, and Welfare
DHHS	Department of Health and Human Services
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	Department of Energy
DOL	Department of Labor
DOT	Department of Transportation
DOT/UN/NA/IMCO	Department of Transportation/United Nations/North America/Intergovernmental Maritime Dangerous Goods Code

DWEL	drinking water exposure level
ECD	electron capture detection
ECG/EKG	electrocardiogram
EEG	electroencephalogram
EEGL	Emergency Exposure Guidance Level
EPA	Environmental Protection Agency
F	Fahrenheit
F_1	first-filial generation
FAO	Food and Agricultural Organization of the United Nations
FDA	Food and Drug Administration
FEMA	Federal Emergency Management Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FPD	flame photometric detection
fpm	feet per minute
FR	Federal Register
FSH	follicle stimulating hormone
g	gram
GC	gas chromatography
gd	gestational day
GLC	gas liquid chromatography
GPC	gel permeation chromatography
HPLC	high-performance liquid chromatography
HRGC	high resolution gas chromatography
HSDB	Hazardous Substance Data Bank
IARC	International Agency for Research on Cancer
IDLH	immediately dangerous to life and health
ILO	International Labor Organization
IRIS	Integrated Risk Information System
Kd	adsorption ratio
kg	kilogram
kkg	metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{50}	lethal concentration, 50% kill
LC_{Lo}	lethal concentration, low
LD_{50}	lethal dose, 50% kill
LD_{Lo}	lethal dose, low
LDH	lactic dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
LT_{50}	lethal time, 50% kill
m	meter
MA	*trans,trans*-muconic acid
MAL	maximum allowable level
mCi	millicurie
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor

MFO	mixed function oxidase
mg	milligram
mL	milliliter
mm	millimeter
mmHg	millimeters of mercury
mmol	millimole
mppcf	millions of particles per cubic foot
MRL	Minimal Risk Level
MS	mass spectrometry
NAAQS	National Ambient Air Quality Standard
NAS	National Academy of Science
NATICH	National Air Toxics Information Clearinghouse
NATO	North Atlantic Treaty Organization
NCE	normochromatic erythrocytes
NCEH	National Center for Environmental Health
NCI	National Cancer Institute
ND	not detected
NFPA	National Fire Protection Association
ng	nanogram
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NIOSHTIC	NIOSH's Computerized Information Retrieval System
NLM	National Library of Medicine
nm	nanometer
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NOES	National Occupational Exposure Survey
NOHS	National Occupational Hazard Survey
NPD	nitrogen phosphorus detection
NPDES	National Pollutant Discharge Elimination System
NPL	National Priorities List
NR	not reported
NRC	National Research Council
NS	not specified
NSPS	New Source Performance Standards
NTIS	National Technical Information Service
NTP	National Toxicology Program
ODW	Office of Drinking Water, EPA
OERR	Office of Emergency and Remedial Response, EPA
OHM/TADS	Oil and Hazardous Materials/Technical Assistance Data System
OPP	Office of Pesticide Programs, EPA
OPPT	Office of Pollution Prevention and Toxics, EPA
OPPTS	Office of Prevention, Pesticides and Toxic Substances, EPA
OR	odds ratio
OSHA	Occupational Safety and Health Administration
OSW	Office of Solid Waste, EPA
OTS	Office of Toxic Substances
OW	Office of Water
OWRS	Office of Water Regulations and Standards, EPA
PAH	polycyclic aromatic hydrocarbon

PBPD	physiologically based pharmacodynamic
PBPK	physiologically based pharmacokinetic
PCE	polychromatic erythrocytes
PEL	permissible exposure limit
pg	picogram
PHS	Public Health Service
PID	photo ionization detector
pmol	picomole
PMR	proportionate mortality ratio
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
PSNS	pretreatment standards for new sources
RBC	red blood cell
REL	recommended exposure level/limit
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
RQ	reportable quantity
RTECS	Registry of Toxic Effects of Chemical Substances
SARA	Superfund Amendments and Reauthorization Act
SCE	sister chromatid exchange
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
SIC	standard industrial classification
SIM	selected ion monitoring
SMCL	secondary maximum contaminant level
SMR	standardized mortality ratio
SNARL	suggested no adverse response level
SPEGL	Short-Term Public Emergency Guidance Level
STEL	short term exposure limit
STORET	Storage and Retrieval
TD_{50}	toxic dose, 50% specific toxic effect
TLV	threshold limit value
TOC	total organic carbon
TPQ	threshold planning quantity
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TWA	time-weighted average
UF	uncertainty factor
U.S.	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey
VOC	volatile organic compound
WBC	white blood cell
WHO	World Health Organization

>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to
%	percent
α	alpha
β	beta
γ	gamma
δ	delta
μm	micrometer
μg	microgram
q_1^*	cancer slope factor
–	negative
+	positive
(+)	weakly positive result
(–)	weakly negative result

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APPENDIX D. INDEX

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TOXICOLOGICAL PROFILE FOR BROMODICHLOROMETHANE

Agency for Toxic Substances and Disease Registry
U.S. Public Health Service

In collaboration with:

U.S. Environmental Protection Agency (EPA)

December 1989

DISCLAIMER

Mention of company name or product does not constitute endorsement by the Agency for Toxic Substances and Disease Registry.

FOREWORD

The Superfund Amendments and Reauthorization Act of 1986 (Public Law 99-499) extended and amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). This public law (also known as SARA) directed the Agency for Toxic Substances and Disease Registry (ATSDR) to prepare toxicological profiles for hazardous substances which are most commonly found at facilities on the CERCLA National Priorities List and which pose the most significant potential threat to human health, as determined by ATSDR and the Environmental Protection Agency (EPA). The lists of the most significant hazardous substances were published in the Federal Register on April 17, 1987, and on October 20, 1988.

Section 110 (3) of SARA directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list. Each profile must include the following content:

(A) An examination, summary and interpretation of available toxicological information and epidemiological evaluations on the hazardous substance in order to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects,

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure which present a significant risk to human health of acute, subacute, or chronic health effects, and

(C) Where appropriate, an identification of toxicological testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

This toxicological profile is prepared in accordance with guidelines developed by ATSDR and EPA. The original guidelines were published in the Federal Register on April 17, 1987. Each profile will be revised and republished as necessary, but no less often than every 3 years, as required by SARA.

The ATSDR toxicological profile is intended to characterize succinctly the toxicological and health effects information for the hazardous substance being described. Each profile identifies and reviews the key literature that

describes a hazardous substance's toxicological properties. Other literature is presented but described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

Each toxicological profile begins with a public health statement, which describes in nontechnical language a substance's relevant toxicological properties. Following the statement is material that presents levels of significant human exposure and, where known, significant health effects. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to protection of public health will be identified by ATSDR, the National Toxicology Program of the Public Health Service, and EPA. The focus of the profiles is on health and toxicological information; therefore, we have included this information in the front of the document.

The principal audiences for the toxicological profiles are health professionals at the federal, state, and local levels, interested private sector organizations and groups, and members of the public. We plan to revise these documents as additional data become available.

This profile reflects our assessment of all relevant toxicological testing and information that has been peer reviewed. It has been reviewed by scientists from ATSDR, EPA, the Centers for Disease Control, and the National Toxicology Program. It has also been reviewed by a panel of nongovernment peer reviewers and was made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

Walter R. Dowdle, Ph.D.
Acting Administrator
Agency for Toxic Substances and
Disease Registry

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1. PUBLIC HEALTH STATEMENT

1.1 WHAT IS BROMODICHLOROMETHANE?

Bromodichloromethane (BDCM) is a colorless, heavy, nonburnable liquid. BDCM does not usually exist as a liquid in the environment. Rather, it usually is found evaporated in air or dissolved in water.

Most BDCM in the environment is formed as a by-product when chlorine is added to drinking water to kill disease-causing organisms. Small amounts of BDCM are also made in chemical plants for use in laboratories or in making other chemicals. A very small amount (less than 1% of the amount coming from human activities) is formed by algae in the ocean.

BDCM evaporates quite easily, so most BDCM that escapes into the environment from chemical facilities, waste sites, or drinking water enters the atmosphere as a gas. BDCM is slowly broken down (about 90% in a year) by chemical reactions in the air. Any BDCM that remains in water or soil may also be broken down slowly by bacteria.

Further information on the properties and uses of BDCM, and how it behaves in the environment, may be found in Chapters 3, 4, and 5.

1.2 HOW MIGHT I BE EXPOSED TO BROMODICHLOROMETHANE?

For most people, the most likely means of exposure to BDCM is by drinking chlorinated water. Usually the levels in drinking water are between 1 and 10 ppb (parts per billion). BDCM is also found in some foods and beverages such as ice cream or soft-drinks that are made using chlorinated water, but this is probably not a major source of exposure. BDCM has been found in chlorinated swimming pools, where exposure might occur by breathing the vapors or through the skin. Exposure to BDCM might also occur by breathing BDCM in the air in or near a laboratory or factory that made or used BDCM. However, BDCM is not widely used in this country, so this is not likely for most people. Average levels of BDCM in air are usually quite low (less than 0.2 ppb). Another place where human exposure might occur is near a waste site where BDCM has been allowed to leak into water or soil. In this situation, people could be exposed by drinking the water or by getting the soil on their skin. BDCM has been found in water and soil at some waste sites (about 1% to 10% of those tested), usually at levels of 1 to 50 ppb. Further information on how people might be exposed to BDCM is given in Chapter 5.

1. PUBLIC HEALTH STATEMENT

1.3 HOW CAN BROMODICHLOROMETHANE ENTER AND LEAVE MY BODY?

Studies in animals show that almost all BDCM swallowed in water or food will enter the body by moving from the stomach or intestines into the blood. It is likely that BDCM would also move from the lungs into the blood if it were breathed in and would cross the skin if skin contact occurred, but this has not been studied. Bromodichloromethane leaves the body mostly by being breathed out through the lungs. Smaller amounts leave in the urine and feces. BDCM removal is fairly rapid and complete (about 95% in 8 hours), so it does not usually build up in the body. Further information on how BDCM enters and leaves the body is given in Chapter 2.

1.4 HOW CAN BROMODICHLOROMETHANE AFFECT MY HEALTH?

The effects of BDCM depend on how much is taken into the body. In animals, the main effect of eating or drinking large amounts of BDCM is injury to the liver and kidneys, These effects can occur within a short time after exposure. High levels can also cause effects on the brain, leading to incoordination and sleepiness. There is some evidence that BDCM can be toxic to developing fetuses, but this has not been well-studied. Studies in animals show that intake of BDCM for several years in food or water can lead to cancer of the liver, kidney and intestines. Although effects of BDCM have not been reported in humans, effects would probably occur if enough BDCM were taken into the body. Further information on how BDCM can affect the health of humans and animals is presented in Chapter 2.

1.5 IS THERE A MEDICAL TEST TO DETERMINE IF I HAVE BEEN EXPOSED TO BROMODICHLOROMETHANE?

Methods are available to measure low levels of BDCM in human blood, breath, urine and fat, but not enough information is available to use such tests to predict if any health effects might result. Because special equipment is needed, these tests are not usually done in doctors' offices. Because BDCM leaves the body fairly quickly, these methods are best suited to detecting recent exposures. Further information on how BDCM can be measured in exposed humans is presented in Chapter 6.

1. PUBLIC HEALTH STATEMENT

1.6 WHAT LEVELS OF EXPOSURE HAVE RESULTED IN HARMFUL HEALTH EFFECTS?

Tables l-l through l-4 show the relationship between exposure to BDCM and known health effects. Minimal Risk Levels (MRLs) are also included in Table 1-3. These MRLs were derived from animal data for both short-term and longer-term exposure, as described in Chapter 2 and in Table 2-l. These MRLs provide a basis for comparison with levels that people might encounter either in food or drinking water. If a person is exposed to BDCM at an amount below the MRL, it is not expected that harmful noncancer health effects will occur. Because these levels are based only on information currently available, some uncertainty is always associated with them. Also, because the method for deriving MRLs does not use any information about cancer, a MRL does not imply anything about the presence, absence or level of risk of cancer. Further information on the levels of BDCM that have been observed to cause health effects in animals is presented in Chapter 2.

1.7 WHAT RECOMMENDATIONS HAS THE FEDERAL GOVERNMENT MADE TO PROTECT HUMAN HEALTH?

The U.S. Environmental Protection Agency (EPA) has set a Maximum Contaminant Level in drinking water of 0.10 ppm (parts per million) for the combination of BDCM and a group of similar compounds (trihalomethanes). Most water samples in the U.S. have BDCM levels lower than this. The Food and Drug Administration (FDA) has set the same limit for bottled water, but no tolerance limits have been set for BDCM in food. Because it has such limited use in industry, there is no Occupational Safety and Health Administration standard for BDCM. Further information on regulations concerning BDCM is presented in Chapter 7.

1.8 WHERE CAN I GET MORE INFORMATION?

If you have further questions or concerns, please contact your State Health or Environmental Department or:

Agency for Toxic Substances and Disease Registry
Division of Toxicology
1600 Clifton Road, E-29
Atlanta, Georgia 30333

1. PUBLIC HEALTH STATEMENT

TABLE 1-1. Human Health Effects from Breathing BDCM*

Short-term Exposure (less than or equal to 14 days)		
Levels in Air (ppm)	Length of Exposure	Description of Effects
		The health effects resulting from short-term human exposure to air containing specific levels of BDCM are not known.
Long-term Exposure (greater than 14 days)		
Levels in Air (ppm)	Length of Exposure	Description of Effects
		The health effects resulting from long-term human exposure to air containing specific levels of BDCM are not known.

* See Section 1.2 for a discussion of exposures encountered in daily life.

TABLE 1-2. Animal Health Effects from Breathing BDCM

Short-term Exposure (less than or equal to 14 days)		
Levels in Air (ppm)	Length of Exposure	Description of Effects
		The health effects resulting from short-term animal exposure to air containing specific levels of BDCM are not known.
Long-term Exposure (greater than 14 days)		
Levels in Air (ppm)	Length of Exposure	Description of Effects
		The health effects resulting from long-term animal exposure to air containing specific levels of BDCM are not known.

TABLE 1-3. Human Health Effects from Eating or Drinking BDCM*

Short-term Exposure (less than or equal to 14 days)		
Levels in Food (ppm)	Length of Exposure	Description of Effects
1.3		Estimated Minimal Risk Level (based on studies in animals; see Section 1.6 for discussion)
Levels in Water (ppm)		The health effects resulting from short-term human exposure to water containing specific levels of BDCM are not known.
Long-term Exposure (greater than 14 days)		
Levels in Food (ppm)	Length of Exposure	Description of Effects
0.6		Estimated Minimal Risk Level (based on studies in animals; see Section 1.6 for discussion)
Levels in Water (ppm)		The health effects resulting from long-term human exposure to water containing specific levels of BDCM are not known.

* See Section 1.2 for a discussion of exposures encountered in daily life.

TABLE 1-4. Animal Health Effects from Eating or Drinking BDCM

Short-term Exposure (less than or equal to 14 days)		
Levels in Food (ppm)	Length of Exposure	Description of Effects
280	14 days	Liver injury in mice.
570	14 days	Kidney injury in mice.
1,000	10 days	Impaired fetal development in rats.
1,200	14 days	Death in mice.
Levels in Water (ppm)		The health effects resulting from short-term animal exposure to water containing specific levels of BDCM are not known.
Long-term Exposure (greater than 14 days)		
Levels in Food (ppm)	Length of Exposure	Description of Effects*
190	2 years	Kidney injury in mice.
380	2 years	Liver injury in mice.
Levels in Water (ppm)		The health effects resulting from long-term animal exposure to water containing specific levels of BDCM are not known.

* These effects are listed at the lowest level at which they were first observed. They may also be seen at higher levels.

2. HEALTH EFFECTS

2.1 INTRODUCTION

This chapter contains descriptions and evaluations of studies and interpretation of data on the health effects associated with exposure to BDCM. Its purpose is to present levels of significant exposure for BDCM based on toxicological studies, epidemiological investigations, and environmental exposure data. This information is presented to provide public health officials, physicians, toxicologists, and other interested individuals and groups with (1) an overall perspective of the toxicology of BDCM and (2) a depiction of significant exposure levels associated with various adverse health effects.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals address the needs of persons living or working near hazardous waste sites, the data in this section are organized first by route of exposure--inhalation, oral, and dermal--and then by health effect--death, systemic, immunological, neurological, developmental, reproductive, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods--acute, intermediate, and chronic.

Levels of significant exposure for each exposure route and duration (for which data exist) are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELS) or lowest-observed-adverse-effect levels (LOAELS) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. These distinctions are intended to help the users of the document identify the levels of exposure at which adverse health effects start to appear, determine whether or not the intensity of the effects varies with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown on the tables and graphs may differ depending on the user's perspective. For example, physicians concerned with the interpretation of clinical findings in exposed persons or with the identification of persons with the potential to develop such disease may be interested in levels of exposure associated with "serious" effects. Public health officials and project managers concerned with response actions at Superfund sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAEL) or exposure levels below which no adverse

effects (NOAEL) have been observed. Estimates of levels posing minimal risk to humans (minimal risk levels, MRLs) are of interest to health professionals and citizens alike;

For certain chemicals, levels of exposure associated with carcinogenic effects may be indicated in the figures. These levels reflect the actual doses associated with the tumor incidences reported in the studies cited. Because cancer effects could occur at lower exposure levels, the figures also show estimated excess risks, ranging from a risk of one in 10,000 to one in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made, where data were believed reliable, for the most sensitive noncancer end point for each exposure duration. MRLs include adjustments to reflect human variability and, where appropriate, the uncertainty of extrapolating from laboratory animal data to humans. Although methods have been established to derive these levels (Barnes et al. 1987; EPA 1980d), uncertainties are associated with the techniques.

2.2.1 Inhalation Exposure

No studies were located regarding the following health effects in humans or experimental animals following inhalation exposure to BDCM:

2.2.1.1 Death

2.2.1.2 Systemic Effects

2.2.1.3 Immunological Effects

2.2.1.4 Neurological Effects

2.2.1.5 Developmental Effects

2.2.1.6 Reproductive Effects

2.2.1.7 Genotoxic Effects

2.2.1.8 Cancer

2. HEALTH EFFECTS

2.2.2 Oral Exposure

No studies were located regarding health effects in humans associated with ingestion of BDCM. Figure 2-l and Table 2-l summarize the health effects observed in experimental animals following oral exposure to BDCM. These effects are discussed below.

2.2.2.1 Death

Most estimates of acute oral LD_{50} values for BDCM in rodents range between 400 and 1000 mg/kg (Aida et al. 1987; Chu et al. 1980; Bowman at al. 1978). Typical pathological changes observed in acutely poisoned animals include fatty infiltration of liver and hemorrhagic lesions in kidney, adrenals, lung and brain (Bowman et al. 1978). In a 14-day repeated-dose study in mice, all animals dosed with 150 mg/kg/day died (NTP 1987). This dose has been converted to an equivalent concentration of 1,200 ppm in food for presentation in Table l-4. Males appear to be slightly more susceptible to the lethal effects of BDCM than females, both in rats (Aida et al. 1987; Chu et al. 1980, 1982a; NTP 1987), and in mice (Bowman et al. 1978; NTP 1987).

The highest NOAEL values and all reliable LOAEL values for death in each species and duration category are recorded in Table 2-l and plotted in Figure 2-l.

2.2.2.2 Systemic Effects

No studies were located regarding effects on the respiratory, cardiovascular, gastrointestinal, musculoskeletal, or dermal systems in humans or animals following oral exposure to BDCM.

Hematological Effects. Hemoglobin and hematocrit were significantly reduced in male rats following a single dose of 390 mg/kg of BDCM (Chu et al. 1982a). The basis of this effect was not investigated. Exposure in drinking water for 90 days to a dose of 213 mg/kg/day caused no effect on lymphocyte levels in either males or females (Chu et al. 1982b). A slight reduction in lymphocyte count was noted in females 90 days after exposure ceased, which the authors felt might be related to endogenous release of steroids. Rats fed BDCM in their diets at intake levels of 130 mg/kg/day for 24 months exhibited no hematological changes compared to controls (Tobe et al. 1982).

TABLE 2-1. Levels of Significant Exposure to BDCM - Oral

Graph Key	Species	(Route)	Exposure Duration/ Frequency [a]	Syst. Effect	NOAEL mg/kg/day	LOAEL (Effect) Less Serious mg/kg/day	LOAEL (Effect) Serious mg/kg/day	Reference
ACUTE EXPOSURE								
Death								
1	rat	(G)	1 dose				430 LD50M 510 LD50F	Aida et al. 1987
2	rat	(G)	1 dose		390		916 LD50M 969 LD50F	Chu et al. 1980
3	mouse	(G)	14 d		75		150 100% died	NTP 1987
4	mouse	(G)	1 dose		300		600	NTP 1987
5	mouse	(G)	1 dose				450 LD50M 900 LD50F	Bowman et al. 1978
Systemic								
6	rat	(G)	10 d	Hepatic		50 liver wt.		Ruddick et al. 1983
7	rat	(G)	14 d	Renal		600 reddened medullae		NTP 1987
8	rat	(G)	1 dose	Hepatic	300	1250 pale color		NTP 1987
9	rat	(G)	1 dose	Renal	390			Chu et al. 1982a
10	rat	(G)	1 dose	Hemato		390 decreased hematocrit		Chu et al. 1982a
11	rat	(G)	1 dose	Hepatic	396	495 GPT	990 GPT	Hewitt et al. 1983
12	mouse	(G)	14 d	Renal	75	150 reddened medullae		NTP 1987
13	mouse	(G)	14 d	Renal		250 BUN		Munson et al. 1982
14	mouse	(G)	14 d	Hepatic		125 fibrinogen		Munson et al. 1982
15	mouse	(G)	14 d	Hepatic		37[b] microscopic lesions		Condie et al. 1983
16	mouse	(G)	14 d	Renal		74 PAH inhib.	148 microscopic lesions	Condie et al. 1983

TABLE 2-1. - continued

Graph Key	Species (Route)	Exposure Duration/ Frequency (a)	Syst. Effect	NOAEL mg/kg/day	LOAEL (Effect) Less Serious mg/kg/day	LOAEL (Effect) Serious mg/kg/day	Reference
17	mouse (G)	14 d	Hepatic		125 liver wt. inc.		Munson et al. 1982
18	mouse (G)	14 d	Renal	300			NTP 1987
Neurological							
19	rat (G)	1 dose				1500 ataxia	Chu et al. 1980
20	rat (G)	14 d			600 hyperactive		NTP 1987
21	mouse (G)	1 dose			273 coordination		Balster and Borzelleca 1982
22	mouse (G)	1 dose			600 lethargy		NTP 1987
23	mouse (G)	14 d		11.6			Balster and Borzelleca 1982
Developmental							
24	rat (G)	10 d d. 6-10 of gest.				50 fetotox.	Ruddick et al. 1983
INTERMEDIATE EXPOSURE							
Death							
25	rat (W)	28 d		120			Chu et al. 1982a
26	mouse (G)	13 wk 5d/wk		100			NTP 1987
Systemic							
27	rat (W)	28 d	Renal	120			Chu et al. 1982a
28	rat (W)	90 d	Hepatic		7 lesions		Chu et al. 1982b
29	rat (W)	28 d	Hepatic	120			Chu et al. 1982a
30	rat (G)	13 wk 5d/wk	Hepatic	150		300 lesions	NTP 1987
31	rat (W)	28 d	Hemato	120			Chu et al. 1982a
32	rat (W)	90 d	Hemato	213			Chu et al. 1982b
33	rat (G)	13 wk 5d/wk	Renal	150		300 lesions	NTP 1987

TABLE 2-1. - continued

Graph Key	Species (Route)	Exposure Duration/ Frequency[a]	Syst. Effect	NOAEL mg/kg/day	LOAEL (Effect) Less Serious mg/kg/day	LOAEL (Effect) Serious mg/kg/day	Reference
34	mouse (G)	13 wk 5d/wk	Hepatic	100		200 lesions	NTP 1987
35	mouse (G)	13 wk 5d/wk	Hepatic	100			NTP 1987
36	mouse (G)	13 wk 5d/wk	Renal	50		100 focal necrosis	NTP 1987
CHRONIC EXPOSURE							
Neurological							
37	mouse (G)	30 d		100			Balster and Borzelleca 1982
38	mouse (G)	90 d		11.6			Balster and Borzelleca 1982
39	mouse (G)	60 d			100 operant behavior		Balster and Borzelleca 1982
Death							
40	rat (G)	104 wk 5d/wk		100			NTP 1987
41	mouse (G)	104 wk 5d/wk		50			NTP 1987
Systemic							
42	rat (G)	104 wk 5d/wk	Hepatic		50 fatty degen.	100 lesions	NTP 1987
43	rat (F)	24 mo	Renal	220			Tobe et al. 1982
44	rat (F)	24 mo	Hemato	220			Tobe et al. 1982
45	rat (G)	104 wk 5d/wk	Renal		50 cytomegaly		NTP 1987
46	rat (F)	24 mo	Hepatic	41	220 GPT		Tobe et al. 1982
47	mouse (G)	104 wk 5d/wk	Renal		25[c] cytomegaly		NTP 1987
48	mouse (G)	104 wk 5d/wk	Renal	150			NTP 1987
49	mouse (G)	104 wk 5d/wk	Hepatic		50 fatty degn.		NTP 1987

TABLE 2-1. - continued

Graph Key	Species	(Route)	Exposure Duration/ Frequency [a]	Syst. Effect	NOAEL mg/kg/day	LOAEL (Effect) Less Serious mg/kg/day	LOAEL (Effect) Serious mg/kg/day	Reference
Cancer								
50	rat	(W)	180 wk 7d/wk				150 CEL (liver tumors)	Tumasonis et al. 1985
51	rat	(G)	104 wk 5d/wk				50 CEL (intestinal carcinoma)	NTP 1987
52	mouse	(G)	104 wk 5d/wk				50 CEL (renal carcinoma)	NTP 1987
53	mouse	(G)	104 wk 5d/wk				75 CEL (liver tumors)	NTP 1987

[a]G = Gavage; W = Drinking Water, F = Feed.
[b]Used to derive acute oral MRL: dose divided by an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).
[c]Used to derive chronic oral MRL: dose adjusted for intermittent exposure and divided by uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability), resulting in an MRL of 0.018 mg/kg/day. This MRL has been converted to an equivalent concentration in food (0.6 ppm) for presentation in Table 1-3.

LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; mg/kg/day = milligram/kilogram/day; (G) = gavage; LD50 = lethal dose, 50% mortality; d = day; wt. = weight; hemato = hematological; GPT = glutamate-pyruvate transaminase; BUN = blood urea nitrogen; PAH = para-amino hippuric acid; inhib. = inhibition; inc. = increased; fetotox = fetotoxicity; gest = gestation; (W) = drinking water; wk = week; degen = degeneration; (F) = food; mo = month; CEL = cancer effect level.

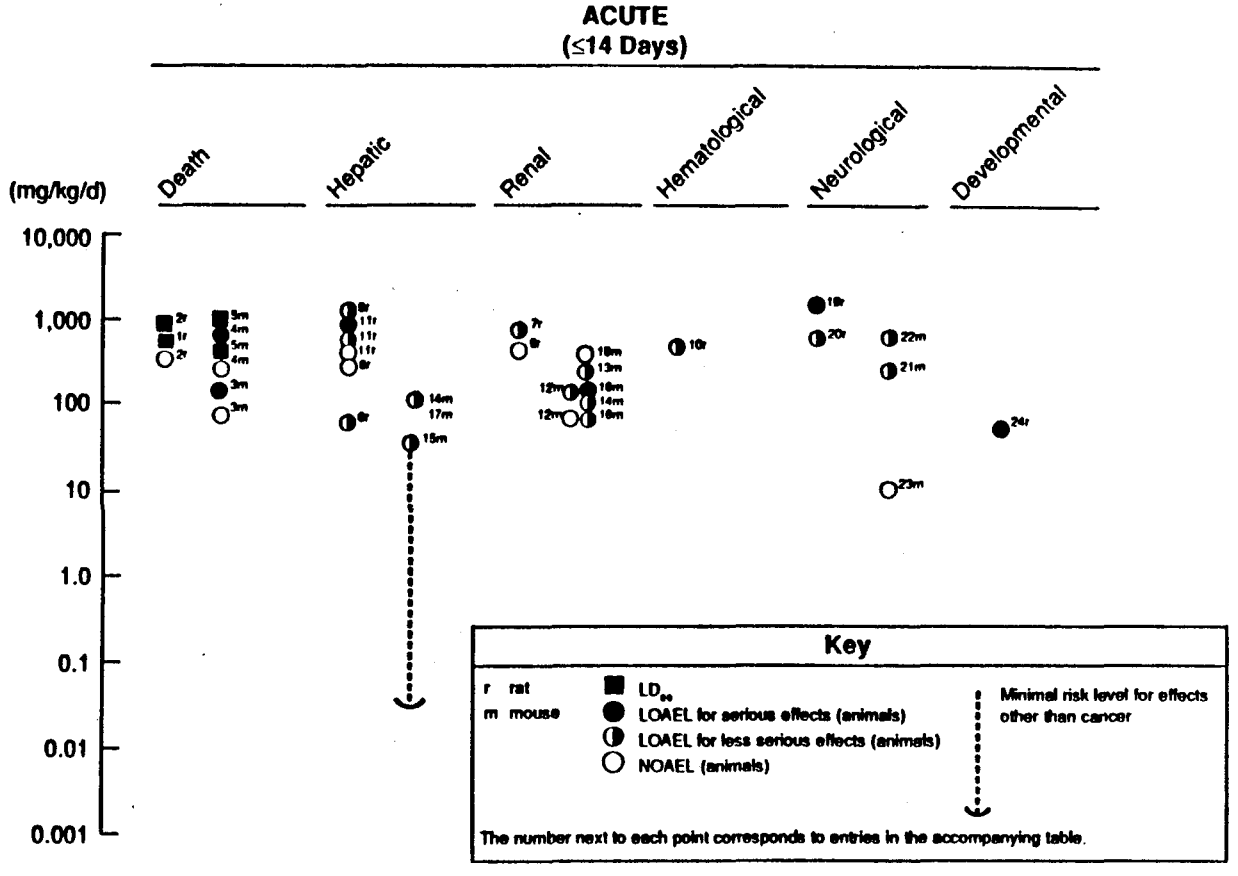


FIGURE 2-1. Levels of Significant Exposure to BDCM – Oral

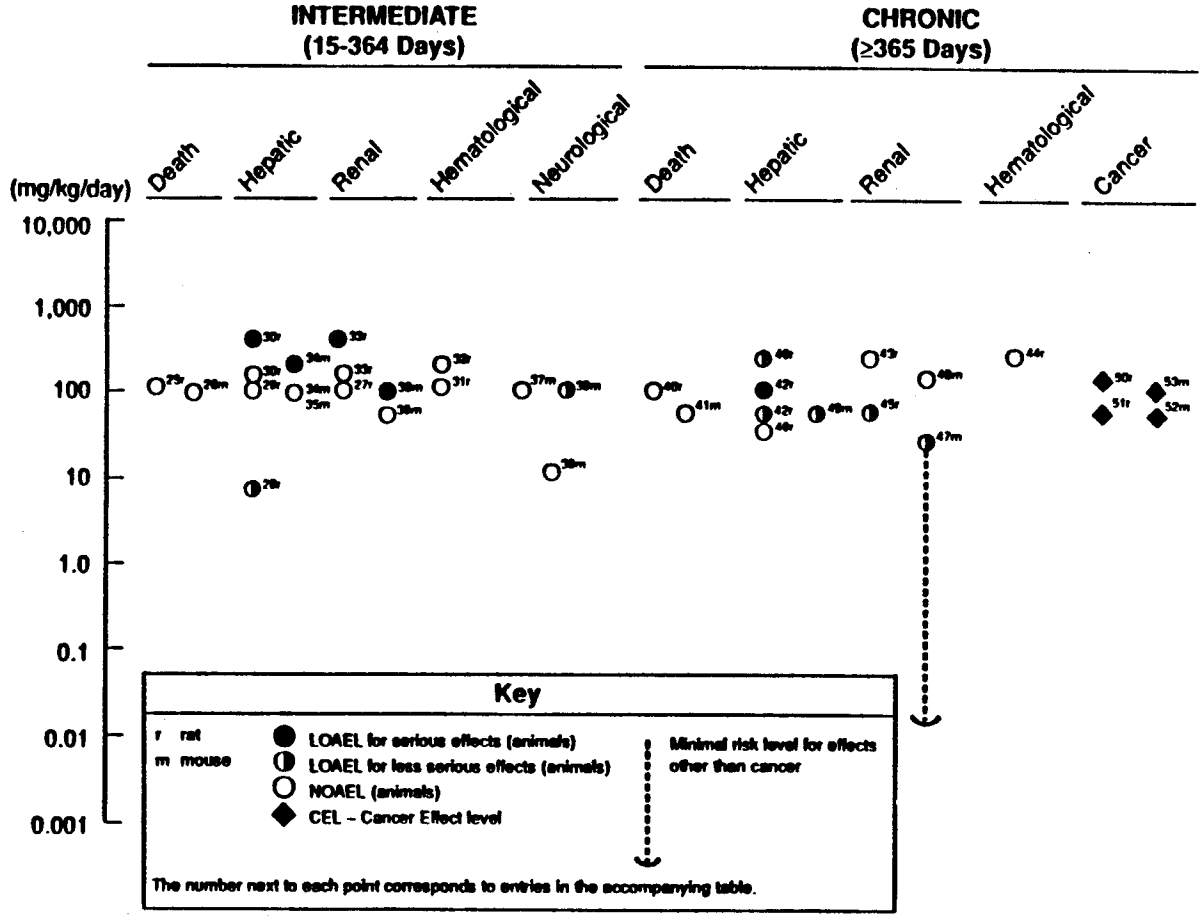


FIGURE 2-1. (con't)

2. HEALTH EFFECTS

Hepatic Effects. A number of studies in animals indicate that the liver is susceptible to injury by BDCM. Typical signs include increased liver weight, pale discoloration, increased levels of hepatic tissue enzymes in serum, decreased levels of secreted hepatic proteins (fibrinogen) in blood, and focal areas of inflammation or degeneration. In acute (single dose) studies, these effects have been noted at doses of around 1,250 mg/kg or higher (NTP 1987). It should be noted that this dose level causes death within two weeks (NTP 1987).

In subchronic studies (10 to 14 days) in mice and rats, mild effects on liver have been noted at doses as low as 37 mg/kg/day (Condie et al. 1983) and 50 mg/kg/day (Ruddick et al. 1983). Effects included slightly increased liver weights (Ruddick et al. 1983) and microscopic changes that were rated as "minimal" (Condie et al. 1983). The effects become more pronounced at doses of 125 to 300 mg/kg/day (Condie et al. 1983; Munson et al. 1982). The dose of 37 mg/kg/day has been converted to an equivalent concentration of 280 ppm in food for presentation in Table 1-4.

Although hepatic effects at doses of 40 to 50 mg/kg/day are minimal, it appears that this is the approximate threshold for the appearance of more marked effects at higher doses, so the dose of 37 mg/kg/day (Condie et al. 1983) has been used to derive the acute MRL for BDCM. Based on this value, an acute oral MRL of 0.037 mg/kg/day was calculated, as described in the footnote in Table 2-1. This MRL has been converted to an equivalent concentration in food (1.3 ppm) for presentation in Table 1-3.

Most longer-term studies report signs of liver injury in rats or mice at doses of 50 to 200 mg/kg/day (Dunnick et al. 1987; NTP 1987; Tobe et al. 1982). These doses are not significantly different from those observed to cause hepatic injury in acute and short-term studies, suggesting that there is a relatively low tendency toward cumulative injury to liver.

An exception is the study of Chu et al. (1982b), where statistically significant effects on liver were noted in rats exposed to doses as low as 7 mg/kg/day for 90 days. However, these effects were minimal (the authors assigned a severity score of 2 on a scale of 1 to 10) and showed essentially no dose-response tendency. Because this observation is of uncertain significance and is inconsistent with NOAEL

estimates from other intermediate and chronic studies, it has not been selected for calculation of a longer-term MRL. The chronic dose level of 50 mg/kg/day (NTP 1987) has been converted to an equivalent concentration of 380 ppm in food for presentation in Table l-4.

The highest NOAEL values and all reliable LOAEL values for hepatic injury in each species and duration category are recorded in Table 2-l and plotted in Figure 2-l.

Renal Effects. Studies in animals reveal that the kidney is also susceptible to injury by BDCM, typically at dose levels similar to those that effect the liver. For example, in 14-day studies, Munson et al. (1982) observed increases in blood urea nitrogen (BUN) in mice dosed with 250 mg/kg/day, and Condie et al. (1983) reported decreased uptake of p-aminohippurate (PAH) into kidney slices from mice dosed with 74 to 148 mg/kg/day. Similarly, Ruddick et al. (1983)observed increased renal weight in rats dosed with 200 mg/kg/day for 10 days. The dose of 74 mg/kg/day has been converted to an equivalent concentration of 570 ppm in food for presentation in Table l-4.

In longer-term studies, areas of focal necrosis were observed in the proximal tubular epithelium in male mice exposed for 13 weeks to doses of 100 mg/kg/day, and cytomegaly was noted following chronic exposure to 25 mg/kg/day (Dunnick et al. 1987; NTP 1987). Female mice were somewhat less susceptible than males. In rats, cytomegaly and nephrosis were observed in both males and females at chronic exposure levels of 50 to 100 mg/kg/day (NTP 1987). The dose of 25 mg/kg/day has been converted to an equivalent concentration of 190 ppm in food for presentation in Table l-4. This dose has also been selected as the most appropriate value for calculation of the chronic MRL for BDCM. Based on this value, a chronic oral MRL of 0.018 mg/kg/day was calculated, as described in the footnote in Table 2-l. This MRL has been converted to an equivalent concentration in food (0.6 ppm) for presentation in Table l-3.

The highest NOAEL values and all reliable LOAEL values for renal injury in each species and duration category are recorded in Table 2-1 and plotted in Figure 2-l.

2.2.2.3 Immunological Effects

The effects of BDCM on the immune system have not been thoroughly studied. Munson et al. (1982) administered BDCM to mice for 14 days, and observed a decrease in female mice in the number of antibody forming

cells in spleen and a decrease in the hemagglutination titer at doses of 125 to 250 mg/kg/day. The authors felt that the humoral immune system may have potential to serve as an early indicator of halomethane toxicity.

2.2.2.4 Neurological Effects

No studies were located regarding histological or electrophysiological effects of BDCM on the nervous system. Rats and mice administered oral doses of 150 to 600 mg/kg often display acute signs of CNS depression, including lethargy, labored breathing, sedation and flaccid muscle tone (NTP 1987; Aida et al. 1987; Balster and Borzelleca 1982; Chu et al. 1980). These effects tend to reverse after a period of several hours.

To determine whether BDCM exposure resulted in any longer-lasting . changes in behavior, Balster and Borzelleca (1982) performed a series of tests in mice 24 hours or more after the last of a series of doses of BDCM. Exposure to doses of 1.2 to 11.6 mg/kg/day for 14 to 90 days had no effect on tests of coordination, strength, endurance or exploratory activity, and 90 days exposure to 100 mg/kg/day did not effect passiveavoidance
learning. Exposure to 100 or 400 mg/kg/day for go-days did result in an acute effect on operant behavior (decreased pressing of a lever that presented food), but this change tended to diminish over the exposure period, suggesting there was no progressive effect and that partial tolerance developed.

The highest NOAEL values and all reliable LOAEL values for neurological effects in each species and duration category are recorded in Table 2-l and plotted in Figure 2-l.

2.2.2.5 Developmental Effects

Ruddick et al. (1983) reported an increased incidence of sternebral anomalies in fetuses from rats that had been exposed to BDCM at doses of 50 to 200 mg/kg/day on days 6 to 15 of gestation (the critical period for organogenesis). No other dose-related visceral or skeletal anomalies were observed. The authors interpreted the sternebral anomalies to be evidence of a fetotoxic (rather than a teratogenic) effect. These doses also resulted in significant maternal toxicity, as evidenced by a 40% reduction in body weight gain. The dose of 50 mg/kg/day has been converted to an equivalent concentration of 1,000 ppm in food for presentation in Table l-4.

2.2.2.6 Reproductive Effects

No studies were located regarding reproductive effects in humans or animals following oral exposure to BDCM.

2.2.2.7 Genotoxic Effects

An increased frequency of sister chromatic exchange (SCE) in mice exposed to BDCM was reported by Morimoto and Koizumi (1983). Statistically significant increases in SCEs in bone marrow cells were observed in animals dosed with 50 or 100 mg/kg/day for 4 days. Mice given the highest dose tested, 200 mg/kg/day for 4 days, died and could not be evaluated for SCEs in bone marrow cells.

2.2.2.8 Cancer

No studies were located regarding carcinogenic effects in humans following chronic oral exposure to BDCM per se. There are several epidemiological studies that indicate there may be an association between ingestion of chlorinated drinking water (which typically contains BDCM) and increased risk of cancer in humans (Gottlieb et al. 1981; Kanarek and Young 1982; Marienfeld et al. 1986), but such studies cannot provide information on whether any effects observed are due to BDCM or to one or more of the hundreds of other byproducts that are also present in chlorinated water.

However, chronic oral studies in animals provide convincing evidence that BDCM is carcinogenic. In rats, increased frequency of liver tumors was observed in females exposed to 150 mg/kg/day for 180 weeks (Tumasonis et al. 1985), and kidney tumors were observed in both males and females exposed to 100 mg/kg/day (NTP 1987; Dunnick et al. 1987). Incidences of renal tumors were 13/50 and 15/50 in males and females, respectively. Tumors of the large intestine were also observed in rats, at incidences of 13/50 and 45/50 in males exposed to 50 and 100 mg/kg/day, and at an incidence of 12/47 in females dosed at 100 mg/kg/day. In mice, renal tumors were observed in males dosed with 50 mg/kg/day, and hepatic tumors were observed in females dosed with 75 or 150 mg/kg/day. Increased intestinal tumors were not observed in mice (Dunnick et al. 1987; NTP 1987).

2.2.3 Dermal Exposure

No studies were located regarding the following toxic effects in humans or animals following dermal exposure to BDCM:

2. HEALTH EFFECTS

2.2.3.1 Death

2.2.3.2 Systemic Effects

2.2.3.3 Immunological Effects

2.2.3.4 Neurological Effects

2.2.3.5 Developmental Effects

2.2.3.6 Reproductive Effects

2.2.3.7 Genotoxic Effects

2.2.3.8 Cancer

2.3 RELEVANCE TO PUBLIC HEALTH

Oral exposure studies in animals identify the central nervous system, the liver, the kidney and the intestine as the principal target tissues of BDCM. Effects on the central nervous system (lethargy, sedation) are observed mostly following large doses, and are likely the result of a direct narcotic or anaesthetic effect similar to other related chemicals (e.g., chloroform, carbon tetrachloride).

Effects on the liver and kidney include increased organ weight, focal areas of inflammation or degeneration, and decreased function. These effects tend to appear in both tissues at roughly similar doses, usually between 25 and 100 mg/kg/day. This indicates that both tissues are approximately equally susceptible to BDCM. The doses which lead to renal and hepatic injury following intermediate or chronic exposure are generally similar to those causing acute effects (e.g., see Figure 2-l), suggesting that there is a relatively low tendency toward cumulative injury for these noncarcinogenic endpoints. This is probably because both the liver and the kidney are able to repair damaged cells or replace dead cells within a short period after exposure.

BDCM exposure has also been observed to result in developmental toxicity (Ruddick et al. 1983). However, data are available only for doses that cause significant maternal toxicity, so it is not possible to judge whether-developmental effects are likely to occur in animals or humans exposed at lower dose levels.

2. HEALTH EFFECTS

The greatest reason for concern with BDCM exposure is evidence from animal studies that BDCM is carcinogenic. Compared with other trihalomethanes (THMs), BDCM causes the widest spectrum of neoplasms in rats and mice, and is the only THM observed to cause intestinal tumors (Dunnick et al. 1987). In addition, BDCM has been found to be mutagenic in some (but not all) in vitro gene mutation and sister chromatid exchange assays (summarized in Table 2-2). BDCM has also been reported to cause increased sister chromatid exchange in bone marrow cells of mice exposed in vivo (Morimoto and Koizumi 1983). These positive carcinogenicity and genotoxicity studies indicate that exposure to BDCM in chlorinated water or near waste sites might contribute to increased risk of cancer in humans.

Several studies indicate that there are differences in susceptibility to BDCM between species and between sexes. With regard to lethality, for example, male mice are more susceptible than female mice, and both male and female mice are more susceptible than rats. Male mice are also more susceptible to the renal effects of BDCM than females, while in rats, males respond to BDCM with renal cytomegaly and females develop nephrosis. Intestinal tumors are observed in both male and female rats, but not in mice. The basis of these differences is not known, but may possibly be attributed to differences in disposition and metabolism of BDCM between sexes and species. That significant differences exist have been demonstrated by Mink et al, (1986) and Smith et al. (1985), as discussed below in Section 2.6.

Because of the differences in dose susceptibility and tissue specificity observed between sexes and species in animal studies, it is difficult to extrapolate the observations in animals to humans. Until an improved understanding of the mechanistic or toxicokinetic basis of these variables is achieved, it is prudent to assume that the same effects observed in animals will be observed in humans ingesting comparable dose levels.

2.4 LEVELS IN HUMAN TISSUES AND FLUIDS ASSOCIATED WITH HEALTH EFFECTS

BDCM was not detected in samples of human fat studied in the National Human Adipose Tissue Survey (NHATS) (EPA 1986c), and was not detected in the blood of 250 patients studied by Antoine et al. (1986).

TABLE 2-2. Genotoxicity of BDCM

End Point	Species/ Test System	Result	Reference
Gene Mutation	Salmonella (4 strains)	Negative, with and without activation	NTP 1987
Gene Mutation	Saccharomyces XVI85-14C reversion	Negative, with activation; weakly positive, without activation	Nestmann and Lee 1985
Gene Mutation	Saccharomyces D7 gene conversion	Negative, with activation; weakly positive, without activation	Nestmann and Lee 1985
Gene Mutation	Mouse lymphoma	Positive, with activation; negative, without activation	NTP 1987
Chromosomal aberrations	Chinese Hamster ovary (CHO) cells	Negative, with and without activation	NTP 1987
Sister Chromatid Exchange	CHO cells	Negative, with and without activation	NTP 1987
Sister Chromatid Exchange	Human lymphocytes	Delayed cell turnover; moderate activity	Morimoto and Koizumi 1983
Sister Chromatid Exchange	Human lymphoid cells	Elevation in frequency of SCE's	Sobti 1984
Sister Chromatid Exchange	Rat liver cells	Increased 50% above control	Sobti 1984

2. HEALTH EFFECTS

A BDCM concentration of 14 ng/ml was found in a blood sample from one resident living near a waste site in New York (Barkley et al. 1980), but the significance of this isolated observation is difficult to judge. No other studies were located regarding levels of BDCM in human tissues and fluids.

2.5 LEVELS IN THE ENVIRONMENT ASSOCIATED WITH LEVELS IN HUMAN TISSUES AND/OR HEALTH EFFECTS

No studies were located regarding the relationship between environmental levels of BDCM and levels of BDCM in human tissues or fluids or the occurrence of any adverse health effects. Epidemiological studies which indicate there may be an association between consumption of chlorinated water (which contains BDCM) and increased risk of cancer are consistent with, but do not establish, the hypothesis that BDCM increases cancer risk in humans, since chlorinated water contains hundreds'of other chemicals as well.

2.6 TOXICOKINETICS

No studies were located regarding BDCM toxicokinetics in humans, but there are limited data from studies in animals. These data are summarized below,

2.6.1 Absorption

2.6.1.1 Inhalation Exposure

No studies were located regarding absorption following inhalation exposure to BDCM. By analogy with other similar chemicals, it seems likely that BDCM would be well absorbed across the lung in both humans and animals.

2.6.1.2 Oral Exposure

Female monkeys, dosed with radioactive BDCM by gavage, excreted 2% of the administered radioactivity in feces, indicating that gastrointestinal'absorption was essentially complete (Smith et al. 1985). In mice, absorption was also rapid and extensive (Mink et al. 1986). Within eight hours of administration, 90% of the administered radioactivity was excreted in urine or expired air, indicating that

absorption was at least 90% complete. In rats, BDCM was not absorbed as readily as in mice and monkeys with only about 60% of the orally administered dose appearing in the expired air and urine (Mink et al. 1986).

2.6.1.3 Dermal Exposure

No studies were located regarding absorption following dermal exposure to BDCM. By analogy with other similar chemicals, it seems likely that BDCM will be absorbed across the skin.

2.6.2 Distribution

2.6.2.1 Inhalation Exposure

No studies were located regarding distribution in humans or animals following inhalation exposure to BDCM.

2.6.2.2 Oral Exposure

When BDCM was administered to rats by gavage, the compound was slow to leave the stomach (Smith et al. 1985). Three hours after administration, 21.5% of the dose was still in the stomach. Fat, muscle, and liver each contained from 1.8 to 2.8% of the dose, with lower levels in other tissues.

2.6.2.3 Dermal Exposure

No studies were located regarding distribution in humans or animals following dermal exposure to BDCM.

2.6.3 Metabolism

Pathways of BDCM metabolism have not been characterized. Studies in mice indicate that carbon dioxide is a major endproduct in that species, accounting for 81% of the administered dose (Mink et al. 1986). In rats, only 14% of the administered dose was expired as carbon dioxide, and 42% as the parent compound (Mink et al. 1986). As discussed previously, toxicology studies in rats and mice showed that BDCM was more toxic to mice than to rats, and it is possible that these toxicokinetic differences in metabolism may contribute to these differences.

2.6.4 Excretion

2.6.4.1 Inhalation Exposure

No studies were located regarding excretion in humans or animals following inhalation exposure to BDCM.

2.6.4.2 Oral Exposure

The major route of excretion of BDCM in rats, mice, and monkeys is expiration through the lung, either as parent BDCM, or as volatile metabolites such as CO_2 (Mink et al. 1986; Smith et al, 1977; Smith et al. 1985). Excretion via the urine accounts for only a minor fraction of the administered dose (1.4% in rats, 2.2% in mice, and 2% to 6% in monkeys) (Mink et al. 1986; Smith et al. 1985).

Fecal excretion in monkeys accounted for less than 2% of the administered dose 72 hours after dosing (Smith et al. 1985). In rats, Smith et al. (1985) found no detectable amounts of radiolabelled BDCM or metabolites in the feces, but the feces were evaluated only up to 6 hours after administration of BDCM. The shortness of the time interval does not give an accurate assessment of the feces as a route of excretion for BDCM, since 37% of the administered dose in the rats was accounted for in the gastrointestinal tract. No data were available on fecal excretion in mice.

The half-life of BDCM in rats and mice was estimated to be 1.5 and 2 hours, respectively (Mink et al. 1986), and the half-life in monkeys was 4 to 6 hours (Smith et al. 1977). This indicates that BDCM is effectively excreted and that tissue accumulation of BDCM is unlikely.

2.6.4.3 Dermal Exposure

No studies were located regarding excretion in humans or animals following dermal exposure to BDCM.

2.7 INTERACTIONS WITH OTHER CHEMICALS

Hewitt et al. (1983) reported that pretreatment of rats with an oral dose of acetone dramatically increased the hepatic and renal toxicity of an oral dose of BDCM given 18 hours later. This is very similar to the well-documented potentiation of CCl_4 by a variety of alcohols, ketones and other chemicals, suggesting that BDCM and CCl_4 may

exert their toxicity through common mechanisms. Because of the widespread use of alcohols and ketones in industry and in consumer products, this sort of potentiation could be quite important.

A study in rats by Wester et al. (1985) evaluated the effects of ingestion of a mixture of 11 halogenated hydrocarbon contaminants of drinking water, including BDCM. No effects were observed after 25 months of exposure, but the doses employed were so low (0.003 to 0.28 mg/kg/day for BDCM) that this observation does not constitute strong evidence that BDCM does not interact with other chemicals.

2.8 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

No studies were located regarding human populations that are unusually susceptible to BDCM. Because BDCM is known to cause liver injury in animals, humans with preexisting liver diseases (e.g. , hepatitis, cirrhosis) may be particularly susceptible to the hepatotoxic effects of BDCM. Likewise, humans with preexisting kidney diseases may be susceptible to BDCM. By analogy with CCl_4, persons who are heavy drinkers and/or take certain drugs that affect the liver may also be particularly susceptible to the effects of BDCM.

2.9 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of BDCM is available. Where adequate information is not available, ATSDR, in cooperation with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine these health effects (and techniques for developing methods to determine such health effects). The following discussion highlights the availability, or absence, of exposure and toxicity information applicable to human health assessment. A statement of the relevance of identified data needs is also included. In a separate effort, ATSDR, in collaboration with NTP and EPA, will prioritize data needs across chemicals that have been profiled.

2. HEALTH EFFECTS

2.9.1 Existing Information on Health Effects of BDCM

As summarized in Figure 2-2, there are no data on the health effects of BDCM in humans. In animals, there are a number of studies of health effects following oral exposure, and information exists for most endpoints except reproduction. However, no animal toxicity data exist for inhalation or dermal exposure to BDCM.

2.9.2 Data Needs

Single Dose Exposure. There are a number of single dose studies in animals by the oral route, and the range of intake doses leading to lethal and most sublethal effects is reasonably well defined. However, the mechanism of toxicity has not been studied. Such studies would be useful in revealing why there are significant differences in susceptibility between males and females, and whether this is pertinent to the evaluation of human health risk from BDCM. Studies by the oral route are likely to be most relevant, but studies of acute inhalation and dermal toxicity would also be useful, since humans may be exposed by these pathways while bathing or swimming.

Repeated Dose Exposure. Existing studies of health effects in animals administered repeated oral doses of BDCM indicate that there is a relatively low tendency toward cumulative toxicity and that chronic noncarcinogenic effects resemble short-term effects. However, the threshold dose for noncarcinogenic effects is not known with certainty. For example, the study by Chu et al. (1982b) identified minimal effects on the liver at doses of 7 mg/kg/day or higher, while other studies (NTP 1987; Tobe et al. 1982) did not detect effects at doses 6- to 20-times higher. Thus, additional studies to define long-term no-effect levels with greater certainty would help improve risk assessments for BDCM.

Chronic Exposure and Carcinogenicity. Several studies have indicated that chronic oral exposure to BDCM increases cancer risk in animals. Tumors were observed in both liver and kidney tissues (known to be target tissues from subchronic studies of this chemical), and tumors were also observed in the large intestines in rats. This is an unusual tumorigenic response in rats, and the basis for the susceptibility of the large intestine is not known. Further studies would be valuable to reveal the basis for this tissue selectivity, and to obtain improved dose-response data to allow reliable quantitative cancer risk assessment.

	Death	SYSTEMIC Acute	SYSTEMIC Intermed.	SYSTEMIC Chronic	Immunologic	Neurologic	Developmental	Reproductive	Genotoxic	Carcinogenic
Inhalation										
Oral										
Dermal										

HUMAN

	Death	SYSTEMIC Acute	SYSTEMIC Intermed.	SYSTEMIC Chronic	Immunologic	Neurologic	Developmental	Reproductive	Genotoxic	Carcinogenic
Inhalation										
Oral	●	●	●	●	●	●	●		●	●
Dermal										

ANIMAL

● Existing Studies

FIGURE 2-2. Existing Information on Health Effects of BDCM

2. HEALTH EFFECTS

Genotoxicity. An in vivo mutagenicity study in mice indicated that BDCM has potential to cause genetic damage, and in vitro studies also suggest that BDCM has genotoxic potential. Additional in vitro and in vivo studies to evaluate the genotoxicity of BDCM and to identify the mechanism of genotoxic damage in intact mammalian cells would be valuable.

Reproductive Toxicity. No studies were located regarding effects of BDCM on reproduction. Multigeneration studies in animals to evaluate effects of BDCM on reproduction would be valuable.

Developmental Toxicity. One study in rats (Ruddick et al. 1983) indicates that BDCM is fetotoxic at doses that cause maternal toxicity, but effects at lower doses were not evaluated. Additional developmental studies at lower doses and in several other species would be helpful in evaluating more fully the potential of BDCM to cause effects on the developing organism.

Immunotoxicity. Limited data from subchronic oral studies in mice indicate that BDCM adversely affects the immune system. However, the data do not define the threshold for the effect with certainty, nor do the data reveal whether the function of the immune system is significantly impaired. Further studies on the immunotoxicity of BDCM in animals would be valuable in establishing the no-effect level and the relevance to human health.

Neurotoxicity. High doses of BDCM affect the CNS like other halocarbons, causing depressed function and anesthesia. Limited data indicate that repeated exposures may lead to transient effects on behavior, but this has not been investigated in detail. Further neurobehavioral,studies using more sensitive operant measures would help define the exposure levels that lead to these effects, and whether any permanent neurological changes occur.

Epidemiological and Human Dosimetry Studies. No epidemiological studies were located regarding human health effects from exposure to BDCM per se. Epidemiological studies of cancer frequency in populations consuming chlorinated drinking water have been performed, but since BDCM levels tend to vary in concert with the levels of other trihalomethanes and numerous other byproducts of disinfection, it is unlikely that studies of this sort will be able to provide information on the risks contributed specifically by BDCM.

2. HEALTH EFFECTS

Biomarkers of Disease. Since no cases of human disease due to BDCM exposure have been reported, it is not possible to identify biomarkers of disease in humans. Assuming that hepatic and renal injury similar to that observed in animals might occur in exposed humans, early signs of these effects could be detected by standard clinical methods such as serum enzyme levels, PAH clearance, and so on. These tests would not be specific for BDCM, however, and would only detect effects after injury to the tissues has occurred. Efforts to identify a sensitive and specific biomarker of BDCM-induced disease would be helpful.

Disease Registries. No disease registry exists for BDCM-induced diseases in humans. Since the effects observed in animals (hepatic and renal injury, cancer of liver, kidney and intestines) are common diseases in humans, it is likely that a registry of individuals with these diseases would contain only a small number of cases that might be attributable solely to BDCM exposure.

Bioavailability from Environmental Media. No studies were located on the relative bioavailability of BDCM in different environmental media. Based on the physical properties of BDCM, it is not expected that bioavailability would vary widely between water, soil, food, and other media. Studies to investigate this would, nevertheless, be helpful.

Food Chain Bioaccumulation. BDCM is biosynthesized by a variety of marine macroalgae, but whether BDCM from this source or other sources enters the food chain has not been studied. While the relatively rapid metabolism and excretion of BDCM in laboratory animals suggest that marked bioaccumulations is not likely, information on BDCM uptake and retention by fish, plants, and other food sources would be helpful.

Absorption, Distribution, Metabolism, and Excretion. Currently there are no toxicokinetic studies on BDCM following inhalation exposure. Consequently, it would be helpful to determine the fraction of BDCM that is absorbed via inhalation and to investigate whether any significant differences in metabolism or retention exist between inhalation and oral exposures. Similarly, there are no toxicokinetic data regarding dermal exposure to BDCM. Although direct dermal contact with concentrated BDCM is unlikely, dermal contact with water containing BDCM is very common. Consequently, information on dermal absorption rates from aqueous solutions would be helpful.

Most toxicokinetic studies were conducted prior to the findings of cancer in the large intestine of male and female rats following chronic

ingestion of BDCM. Since tumors in the gastrointestinal tract are uncommon in rats, additional toxicokinetic studies focusing on BDCM metabolism and distribution in this tissue would be valuable in understanding the metabolic pathways for BDCM and how the metabolism may be related to the mechanism of toxicity and carcinogenicity of BDCM.

Detailed studies of the enzymic pathways of BDCM metabolism and of the intermediates formed would also be valuable. Metabolic activation to yield highly toxic intermediates is known to be a critical step in the toxicity of some similar compounds (e.g., CCl_4). Investigations to determine whether similar pathways are involved in BDCM toxicity might help resolve many of the special aspects of the toxicity of this compound.

Comparative Toxicokinetics. Since BDCM toxicity appears to differ significantly between sexes and species, additional toxicokinetic studies in several species would be valuable. Such studies would aid in understanding the differences in toxicity between species, and could help identify the most appropriate animal species for use as a model for humans.

2.9.3 On-going Studies

Dr. James Mathews (Research Triangle Institute) is currently performing studies of the dose-dependency of absorption, metabolism and clearance of BDCM following oral exposure of rodents. This research is sponsored by the National Toxicology Program.

3. CHEMICAL AND PHYSICAL INFORMATION

3.1 CHEMICAL IDENTITY

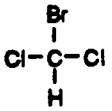
Table 3-l lists common synonyms, trade names and other pertinent identification information for BDCM.

3.2 PHYSICAL AND CHEMICAL PROPERTIES

Table 3-2 lists important physical and chemical properties of BDCM.

3. CHEMICAL AND PHYSICAL INFORMATION

TABLE 3-1. Chemical Identity of BDCM

	Value	References
Chemical Name	Bromodichloromethane	NLM 1988
Synonyms	Dichlorobromomethane; Monobromodichloromethane; Methane, bromodichloro-	NLM 1988
Trade Name (s)	--[a]	
Chemical Formula	$CHBrCl_2$	Verschueren 1977
Chemical Structure		EPA 1983
Identification Numbers:		
CAS Registry	75-27-4	NLM 1988
NIOSH RTECS	PA5310000	HSDB 1988
EPA Hazardous Waste	ND	HSDB 1988
OHM-TADS	ND	HSDB 1988
DOT/UN/NA/IMCO Shipping	ND	HSDB 1988
HSDB	4160	NLM 1988
NCI	C55243	NLM 1988

CAS - Chemical Abstracts Service
NIOSH - National Institute for Occupational Safety and Health
RTECS - Registry of Toxic Effects of Chemical Substances
OHM-TADS - Oil and Hazardous Materials/
Technical Assistance Data System
DOT/UN/NA/IMCO - Department of Transportation/United Nations/North America/
International Maritime Dangerous Goods Code
HSDB - Hazardous Substances Data Bank
NCI - National Cancer Institute

[a]No data located

3. CHEMICAL AND PHYSICAL INFORMATION

TABLE 3-2. Physical and Chemical Properties of BDCM

Property	Value	References
Molecular weight	163.83	Weast 1985
Color	colorless	Verschueren 1977
Physical State	liquid	Verschueren 1977
Melting point, °C	-57.1	Weast 1985
Boiling point, °C	90	Weast 1985
Density, 20/4	1.980	Weast 1985
Odor	ND[a]	
Odor threshold Water Air	ND	
Solubility Water, mg/L	4,500	Mabey et al. 1982
Organic Solvents	soluble	Weast 1985
Partition coefficients Log octanol/water	2.1	Mabey et al. 1982
Log k_{oc}	1.8	Mabey et al. 1982
Vapor Pressure, mm Hg (20°C)	50	Mabey et al. 1982
Henry's Law Constant, atm m^3/mol	2.41E-03	Mabey et al. 1982
Autoignition temperature, °C	ND	
Flash point	ND	
Flammability limits	ND	
Conversion Factors ppm (v/v) to mg/m^3 in air (20 °C)	1 ppm = 6.70 mg/m^3	Verschueren 1977
mg/m^3 to ppm (v/v) in air (20 °C)	1 mg/m^3 = 0.15 ppm	

[a] No data located.

4. PRODUCTION, IMPORT, USE, AND DISPOSAL

4.1 PRODUCTION

BDCM is produced commercially by the reaction of dichloromethane with aluminum bromide. Small quantities of BDCM are currently produced in the United States, but quantitative production volumes are not available.

4.2 IMPORT

No data on imports or exports of BDCM were located. Little, if any, of either is expected.

4.3 USE

In the past, BDCM has been used as a solvent for fats, waxes, and resins, as a flame retardant, as a heavy liquid for mineral and salt separations, and as a fire extinguisher fluid ingredient (Sax 1984). At present, the principal use of BDCM is as a chemical intermediate for organic synthesis and as a laboratory reagent (Sittig 1985; Verschueren 1983). BDCM is not listed as a current ingredient in fire extinguishers, solvents or other commercial products (Gosselein et al. 1984).

4.4 DISPOSAL

Bromodichloromethane is categorized as a hazardous waste constituent (40 CFR 261 App. VIII) and, therefore, must be disposed of in accordance with RCRA regulations. Acceptable disposal methods include incineration using liquid injection, rotary kiln or fluidized bed techniques. At the present time, land disposal of BDCM is also permitted, although trihalomethanes are being evaluated for land disposal prohibition.

BDCM has been detected in the raw and treated wastewater of numerous industries (EPA 1983), but no quantitative data on amounts of BDCM disposed of to the environment were located. BDCM has been detected at 7% of chemical waste sites investigated under Superfund (CLPSD 1988).

4. PRODUCTION, IMPORT, USE AND DISPOSAL

4.5 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of BDCM is available. Where adequate information is not available, ATSDR, in cooperation with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine these health effects (and techniques for developing methods to determine such health effects). The following discussion highlights the availability, or absence, of exposure and toxicity information applicable to human health assessment. A statement of the relevance of identified data needs is also included. In a separate effort, ATSDR, in collaboration with NTP and EPA, will prioritize data needs across chemicals that have been profiled.

4.5.1 Data Needs

Production, Use, Release and Disposal. The minimal commercial use of BDCM is reflected in the absence of available production data. Data on current uses and disposal practices would be valuable in determining whether industrial activities pose an important source of human exposure to BDCM.

According to the Emergency Planning and Community Right to Know Act of 1986(EPCRTKA), (§313), (Pub. L. 99-499, Title III, §313), industries are required to submit release information to the EPA. The Toxic Release Inventory (TRI), which contains release information for 1987, became available in May of 1989. This database will be updated yearly and should provide a more reliable estimate of industrial production and emission.

5. POTENTIAL FOR HUMAN EXPOSURE

5.1 OVERVIEW

The major source of BDCM in the environment is formation as a byproduct during chlorination of water, and many people are exposed to low levels of BDCM in their drinking water. Industrial use of BDCM is sufficiently limited that exposures to industrial emissions outside the workplace are not expected to be of general concern. BDCM has been detected in water and soil at some chemical waste sites, and human exposure may potentially occur in such cases.

BDCM is a volatile chemical, so most of the BDCM that is formed in water or released by industry tends to evaporate into air. BDCM does not adsorb strongly to soils or sediments, nor does it tend to bioaccumulate in fish or other animals.

In the atmosphere, BDCM is thought to undergo slow destruction through oxidative pathways, with a half-life of about two to three months. BDCM remaining in soil or water may undergo microbial degradation. However, these fate processes have not been studied in detail.

5.2 RELEASES TO THE ENVIRONMENT

5.2.1 Air

No studies were located regarding industrial release of BDCM into air. Because of the low volume of BDCM currently in use, it is expected that releases from industrial activities are probably small.

5.2.2 Water

The principal source of BDCM in the environment is from chlorination of water. EPA (1980a) estimated that over 800 kkg (1 kkg = 1 metric ton) are produced annually in this way. It is presumed that essentially all of this is ultimately released into the environment, mainly through volatilization. This may occur either indoors (e.g., while showering, washing, cooking, etc.) or outdoors after discharge of the water to the surface.

5. POTENTIAL FOR HUMAN EXPOSURE

Class et al. (1986) observed trace levels (<l parts per trillion (ppt)) of BDCM and other brominated methanes in seawater and in the air above the ocean at several locations in the Atlantic. The presence of BDCM can be attributed to biosynthesis and release of BDCM by macroalgae (Class et al. 1986; Gschwend et al. 1985). BDCM from this source accounts for less than 1% of the anthropogenic burden of bromomethanes in the atmosphere (Class et al. 1986).

BDCM has been detected in wastewater from a number of industrial discharges and municipal wastewater treatment facilities, usually at concentrations between 1 and 100 μg/L (Staples et al. 1985; Perry et al. 1979; Dunovant et al. 1986). These levels of BDCM are similar to those found in many chlorinated drinking water supplies (see Section 5.4.2, below), and probably most discharges of this sort do not represent a major source of BDCM release to the environment.

5.2.3 Soil

No studies were located regarding release of BDCM to soil.

5.3 ENVIRONMENTAL FATE

5.3.1 Transport and Partitioning

Because of the relatively high vapor pressure of BDCM (50 mm Hg at 20°C), the principal transport process in the environment is volatilization (Class et al. 1986; Gschwend et al. 1985). Over 99% of all BDCM in the environment is estimated to exist in air (EPA 1980a).

Volatilization from surface waters depends on factors such as turbulence and temperature. The volatilization half-life from rivers and streams has been estimated to range from 33 minutes to 12 days, with a typical half-life of 35 hours (Kaczmar et al. 1984). Volatilization rates from surface soils have not been studied in detail, but Wilson et al. (1981) found that about 50% of BDCM applied to a soil column in the laboratory escaped by volatilization.

BDCM may be removed from air by washout in rainfall (Class et al. 1986), but the average rate of this transport process has not been estimated. It is expected that BDCM removed from air in this way would be largely returned to air through volatilization.

5. POTENTIAL FOR HUMAN EXPOSURE

BDCM is moderately soluble in water (4,500 mg/L), and significant transport of BDCM can occur in water, especially in groundwater where volatilization is restricted. This transport pathway may be important at waste sites or other locations where BDCM spills lead to groundwater contamination.

In soil, the relatively low log octanol-water partition coefficient (Kow) indicates that adsorption is not likely to be a dominant factor, and that BDCM spilled into soil will be relatively mobile and may migrate into groundwater (EPA 1985a; Piet et al. 1981). In support of this, Wilson et al. (1981) found that BDCM was not significantly retarded during percolation through a column of sandy soil.

The moderate solubility and low log Kow indicate that bioaccumulation of BDCM by fish or other aquatic species is likely to be minor, but no estimate of a bioaccumulation factor in aquatic species was located.

5.3.2 Transformation and Degradation

5.3.2.1 Air

Pathways responsible for BDCM destruction in the atmosphere are not well studied, but probably involve oxidative reaction with hydroxyl radicals or singlet oxygen (EPA 1980a; Mabey et al. 1982). Direct photochemical decomposition is not likely to be significant (EPA 1980a). The typical atmospheric lifetime of BDCM has been estimated to be two to three months (EPA 1980a). This relatively persistent tropospheric halflife of BDCM suggests that a small percentage of the BDCM present in air will eventually diffuse into the stratosphere where it will be destroyed by photolysis. In addition, long-range global transport is possible.

5.3.2.2 Water

Hydrolysis of BDCM in aqueous media is very slow, with an estimated rate constant at neutral pH of 5.76×10^{-8} hr^{-1} (Mabey et al. 1982). This corresponds to a half-life of more than 1,000 years.

Biodegradation in aqueous media may be significant in some cases. For example, Tabak et al. (1981) reported 35% transformation after seven days incubation in a medium inoculated with sewage. Repeated culturing lead to increased losses, indicating gradual adaptation of the degradative microbes.

5. POTENTIAL FOR HUMAN EXPOSURE

Under aquatic conditions where volatilization cannot occur, biodegradation may be the predominant mechanism for degradation of BDCM. Bouwer et al. (1981) and Bouwer and McCarty (1983a) studied the degradation of BDCM under aerobic and anaerobic conditions in both static and continuous flow systems inoculated with mixed methanogenic bacterial cultures from sewage. Degradation was found to be very limited under aerobic conditions, but essentially complete within 2 days under anaerobic conditions. Slow degradation (50% to 70% in 16 weeks) occurred in sterile media, indicating that a chemical mechanism (hypothesized to be reductive dehalogenation) was operative in addition to the rapid microbial degradation. Microbial degradation was also observed under anaerobic conditions in media inoculated with denitrifying bacteria (Bouwer and McCarty 1983b).

5.3.2.3 Soil

Biodegradation of BDCM in soil has not been studied, but studies in aqueous media indicate that biodegradation might occur under anaerobic conditions (Bouwer et al. 1981; Bouwer and McCarty 1983a, 1983b; Tabak et al. 1981). This suggests that, in regions of soil where volatilization is restricted, biodegradation could be a major removal process.

5.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

5.4.1 Air

Ambient air monitoring data compiled by Brodzinsky and Singh (1983) identified BDCM at four of six sites investigated. Concentrations ranged from 0.76 to 180 ppt, with a mean value of 1.1 ppt. BDCM levels from four sites monitored in California were reported to range from 20 to 100 ppt (Shikiya et al. 1984). BDCM was not detected in a survey of bromine-containing gasses in the atmosphere at the South Pole (Rasmussen and Khalil 1984), although trace levels (1 ppt) were detected in air at several locations in the Atlantic Ocean (Class et al. 1986). This was judged by the authors to be due to releases from macroalgae.

5.4.2 Water

BDCM occurs in water primarily as a by-product of chlorination. Surveys of BDCM levels in chlorinated public drinking water systems across the United States have revealed that BDCM is present in most

systems at concentrations averaging around 1 to 20 μg/L, but ranging up to 125 μg/L in some cases (Coleman *et* al. 1975; EPA 1979; Furlong and D'itri 1986; Symons et al. 1975).

The concentration of BDCM in chlorinated water depends on reaction conditions during the chlorination process. Important parameters include temperature, pH, bromide ion concentration in the source water, fulvic and humic substance concentration in the water, and the chlorination treatment practices (EPA 1985b). The amount of BDCM tends to increase as a function of increasing organic content and bromide ion in the source water (Bellar et al. 1974; Argue110 et al. 1979). Studies by Brett and Calverly (1979) and Argue110 et al. (1979) indicate that BDCM levels increased by 30 to 100% in water system distribution pipes, presumably because formation continues as long as a chlorine residual and organic precursors remain.

BDCM is also formed in chlorinated swimming pools. Beech et al. (1980) measured THM levels in several swimming pools in Miami, and found total THM concentrations averaged from 120 to 660 μg/L. In freshwater pools, most of the total THM was chloroform, with BDCM levels ranging from 13 to 34 μg/L. In saltwater pools, bromoform was the principal THM present, and BDCM concentrations were roughly the same as in freshwater pools.

Monitoring studies of groundwater and surface water at chemical waste sites indicate that BDCM is a relatively infrequent contaminant. BDCM was detected at only 4 of 818 sites on the National Priority List (NPL) , and at 7% of a number of other sites being investigated under Superfund (CLPSD 1988). The average concentration of BDCM in groundwater at these sites was 30 μg/L. Quantitative data for surface water were not available.

5.4.3 Soil

No studies were located on BDCM levels in ambient soil. Because of its volatility, it is likely that BDCM would be present only at low levels in most soils. BDCM was detected in 2% of soil samples taken near chemical waste sites being investigated under Superfund (CLPSD 1988), but quantitative estimates of soil concentration are not available.

5.4.4 Other Media

BDCM is not a common contaminant of food, occurring only in trace quantities in some samples. A market basket study of 39 food items detected BDCM in one dairy composite at 1.2 ppb and in butter at 7 ppb (Entz et al. 1982). A study of BDCM in food processing water and processed foods revealed no detectable levels except in ice cream at one processing plant (0.6 to 2.3 ppt) (Uhler and Diachenko 1987). Soft drinks have been found to contain BDCM (Abdel-Rahman 1982; Entz et al. 1982), but usually at concentrations (0.1 to 6 μg/L) below those found in municipal water supplies. Cooking foods in water containing BDCM is unlikely to lead to contamination, since BDCM would rapidly volatilize (Kool et al. 1981).

BDCM is biosynthesized by marine macroalgae, and has been measured in these organisms at 7-22 ng/g dry weight (Gschwend et al. 1985). Whether BDCM enters and accumulates in the food chain from this source appears to be unlikely, but has not been studied.

5.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

The estimated exposure of the general human population to BDCM from drinking water, assuming a median BDCM concentration of 0.014 mg/L and a water intake for an adult of 2.18 L/day, would be 0.03 mg/day (EPA 1980a). Low levels of exposure might also occur by inhalation of BDCM volatilized from chlorinated water (e.g., while showering, cooking, or swimming), or by dermal contact with such water. Based on a chemical structure analogy to chloroform, an estimated dermal exposure to BDCM in a child swimming two hours/day in a saline pool would typically be 0.003 mg/day, with a maximum of 0.04 mg/day (Beech 1980). Higher exposure levels might occur through ingestion of water contaminated with BDCM near a waste site, but available data suggest that this is not a common occurrence.

No studies were located on human exposure levels in the workplace.

5.6 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

The environmental medium most likely to be contaminated with BDCM is chlorinated water, so any person with above-average contact with such water could have above-average exposures. This includes individuals-who drink very large quantities of water, such as diabetics, workers in hot climates, and so on. It may also include persons with swimming pools or saunas, where exposure could occur by inhalation (especially if the pool

or sauna is indoors) or by dermal contact. Since BDCM levels depend on the organic content of the source water before chlorination, persons whose water source is high in organics are likely to have finished water with higher-than-average BDCM levels.

People working in chemical plants or laboratories where BDCM is made or used would also have potentially high exposures to the chemical, most likely by inhalation exposure. Persons living near waste sites may have potentially high exposure to BDCM, but this can only be evaluated on a case-by-case basis.

5.7 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of BDCM is available. Where adequate information is not available, ATSDR, in cooperation with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine these health effects (and techniques for developing methods to determine such health effects). The following discussion highlights the availability, or absence, of exposure and toxicity information applicable to human health assessment. A statement of the relevance of identified data needs is also included. In a separate effort, ATSDR, in collaboration with NTP and EPA, will prioritize data needs across chemicals that have been profiled.

5.7.1 Data Needs

Physical and Chemical Properties. The physical and chemical properties of BDCM have been determined (see Table 3-2), and further studies on these parameters do not appear to be essential.

Environmental Fate. There are very few quantitative data on the environmental fate and transport of BDCM, and most evaluations are based, entirely or in part, on extrapolations from studies of other similar compounds such as chloroform. Consequently, studies to obtain reliable quantitative rate values for the key fate processes of BDCM would be valuable. Of particular importance would be studies on the volatilization of BDCM from chlorinated drinking water, and on the atmospheric reactions of BDCM. Studies of chemical and biological transformation and degradation rates in soil and water under conditions comparable to those around waste sites would also be helpful.

5. POTENTIAL FOR HUMAN EXPOSURE

Exposure Levels in Environmental Media. Data are available on BDCM levels in drinking water and on how these levels depend on water organic content and treatment conditions. Nevertheless, continued monitoring will be valuable in revealing whether changes in drinking water treatment and disinfection procedures are effective in reducing levels of BDCM and other contaminants.

Studies of BDCM levels in air (especially indoor air) in the vicinity of open bodies of chlorinated water would also be helpful. This would include water treatment plants, swimming pools, and perhaps even home bathtubs or showers. In view of the ready volatilization of BDCM from water, airborne levels in such locations might be significant.

Further monitoring of groundwater, soil and ambient air in the vicinity of chemical waste sites is also needed to determine whether emissions of BDCM from such sites adds significantly to total BDCM exposure.

Exposure Levels in Humans. Current data on BDCM levels in air are not adequate to estimate inhalation exposure in ambient air or the workplace. Collection of such information would be helpful in evaluating the relative contribution of this exposure pathway to the total intake of BDCM. Similar data would be useful for airborne levels of BDCM around swimming pools (especially indoor pools). Data on the presence of BDCM in drinking water appears to be adequate for estimating exposure from consumption of water immediately after taking it from the tap. However, it would be helpful to know how rapidly the BDCM would volatilize from a glass of water or from a bathtub full of water, and what concentration would then be in the breathing zone of occupants of the house.

Exposure Registries. No registry exists for humans known to have been exposed to BDCM. Although exposure to BDCM through drinking water is common, a registry of humans exposed in this way is not likely to help identify BDCM-related diseases in humans, since exposure to BDCM in water are usually low and typically involve exposure to other trihalomethanes and many other byproducts of disinfection as well. A registry of individuals exposed to BDCM during manufacture or use of this chemical might be helpful in identifying possible health effects in humans, although the size of the exposed population is believed to be small.

5. POTENTIAL FOR HUMAN EXPOSURE

5.7.2 On-going Studies

No information was located on any on-going studies on the potential for human exposure to BDCM.

As part of the Third National Health and Nutrition Evaluation Survey (NHANES III), the Environmental Health Laboratory Sciences Division of the Center for Environmental Health and Injury Control, Centers for Disease Control, will be analyzing human blood samples for BDCM and other volatile organic compounds. These data will give an indication of the frequency of occurrence and background levels of these compounds in the general population.

6. ANALYTICAL METHODS

6.1 BIOLOGICAL MATERIALS

As a volatile organohalide, BDCM may be measured with good sensitivity and specificity using gas-chromatographic methods employing halide-specific or election-capture detection. Methods available for separation of BDCM from biological samples prior to analysis include headspace analysis, purge-and-trap collection, solvent extraction, and direct collection on resins.

Headspace analysis offers speed, simplicity, and good reproducibility for a particular type of sample. However, partitioning of the analyte between the headspace and the sample matrix is dependent upon the nature of the matrix and must be determined separately for different kinds of matrices (Walters 1986).

Purge-and-trap collection is well suited to biological samples that are soluble in water (Peoples et al. 1979), and is readily adapted from techniques that have been developed for the analysis of BDCM and other VOCs in water and wastewater. This method consists of bubbling inert gas through a small volume of the sample and collecting the vapor in a trap packed with sorbent. The analytes are then removed from the trap by heating it and backflushing the analytes onto a gas chromatographic column (Pankow et al. 1988). The two materials most widely used for adsorption and thermal desorption of volatile organic compounds collected by the purge-and-trap technique are Carbotrap, consisting of graphitized carbon black, and Tenax,a porous polymer of 2,6-diphenyl-p-phenylene oxide (Fabbri et al. 1987).

Solvent extraction of volatile components from biological fluids is usually performed using dimethyl ether (Zlatkis and Kim 1976). Homogenization of tissue with the extractant and lysing of cells improves extraction efficiency. When, as is often the case, multiple analytes are being determined using solvent extraction, selective extraction and loss of low-boiling compounds can cause errors. Supercritical fluid extraction using pure carbon dioxide or carbon dioxide with additives offers some exciting potential for the extraction of organic analytes such as BDCM from biological samples (Hawthorne 1988).

Analytical methods for the determination of BDCM in biological samples are given in Table 6-1.

6. ANALYTICAL METHODS

TABLE 6-1. Analytical Methods for BDCM in Biological Samples

Sample type	Extraction/cleanup	Detection	Limit of detection	References
Adipose tissue	Purge from liquified fat at 115°C, trap on silica gel, thermal desorption	GC/HSD	<0.8 μg/L	Peoples et al. 1979
Bile acids	NR	GC/ECD	NR	Brechbuehler et al. 1977
Breath, blood, and urine	NR	GC/MS	NR	Barkley et al. 1980
Blood serum	Purge from water-serum mixture containing antifoam reagent at 115°C, trap on Tenax/ silica gel, thermal desorption	GC/HSD	<0.8 μg/L	Peoples et al. 1979
Biofluids[a]	Dilute with water, sealed vial, collection of headspace vapors	GC/ECD	NR	Suitheimer et al. 1982
Grain[b]	Extract with acetone/ water (5/1), dry, inject acetone solution	GC/ECD	NR	AOAC 1984

Abbreviations: GC = gas chromatography; HSD = halide selective detector; ECD = electron capture detector; NR = not reported; MS = mass spectrometry.

[a]This method for volatiles by headspace chromatography can be adapted to bromodichloromethane although the procedure does not list it specifically as an analyte.

[b]Method for carbon tetrachloride, but applicable to bromodichloromethane because of their similar properties.

6. ANALYTICAL METHODS

6.2 ENVIRONMENTAL SAMPLES

BDCM may be isolated from environmental samples using the same methods and principles used for biological samples, followed by gas chromatographic analysis. The most convenient procedure for most liquid and solid samples is the purge-and-trap method. A similar procedure is used for air, involving passing the air through an adsorbent canister, followed by thermal desorption.

Analytical methods for the determination of BDCM in environmental samples are given in Table 6-2.

6.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of BDCM is available. Where adequate information is not available, ATSDR, in cooperation with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine these health effects (and techniques for developing methods to determine such health effects). The following discussion highlights the availability, or absence, of exposure and toxicity information applicable to human health assessment. A statement of the relevance of identified data needs is also included. In a separate effort, ATSDR, in collaboration with NTP and EPA, will prioritize data needs across chemicals that have been profiled.

6.3.1 Data Needs

Methods for Determining Parent Compounds and Metabolites in Biological Materials. Methods are available for the determination of BDCM in biological samples, but there is a need for development of validated standard methods of analysis with well-defined limits of detection, such as those that exist for water and wastewater (EPA 1982a, EPA 1982b) and for solid wastes (EPA 1986a, EPA 1986b).

Animal studies show that BDCM is excreted via the lungs as parent compound or carbon dioxide. Small amounts of carbon monoxide have also been measured in animals after administration of BDCM (Anders et al. 1978). Other metabolites of BDCM have not been identified. Since carbon dioxide and carbon monoxide are not specific to BDCM, measurement of these metabolites is not likely to provide a good index of BDCM exposure.

TABLE 6-2. Analytical Methods for BDCM in Environmental Media

Sample type	Extraction/cleanup	Detection	Limit of detection	References
Air[a]	Coconut shell charcoal sorption carbon disulfide desorption	GC/FID	10 μg per sample	NIOSH 1984
Air	Sorption	GC/CLMD	$3x10^{-13}$ g/sec	Yamada et al. 1982
Air and water	NR	GC/MS	NR	Barkley et al. 1980
Drinking water	Purge and trap	GC/MWED	<1 μg/L	Quimby et al. 1979
Water	Purge and trap	GC/MS	10 μg/L	EPA 1980c
Water	Purge and trap	GC/HSD	0.12 μg/L	EPA 1982a
Water	Purge and trap	GC/MS	2.2 μg/L	EPA 1982b
Water	Purge and trap	GC/HSD	0.5 μg/L	APHA 1985a
Water	Purge and trap	GC/MS	<0.27 μg/L	APHA 1985b
Water	Solvent extraction (isooctane)	GC/HSD	1 μg/L	ASTM 1988
Contaminated soil	Purge and trap	GC/HSD	1.0 μg/kg	EPA 1986a
Wastes, non-water miscible	Purge and trap	GC/HSD	125 μg/kg	EPA 1986a
Solid waste	Purge and trap	GC/MS	5 μg/kg	EPA 1986b

Abbreviations: GC = gas chromatography; FID = flame ionization detector; CLMD = chemiluminescence detection; NR = not reported; MS = mass spectrometry; MWED = microwave emission detector; HSD = halogen-specific detector.

[a]This method for halogenated hydrocarbons can be adapted to bromodichloromethane although the procedure does not list it specifically as an analyte.

6. ANALYTICAL METHODS

Methods for Biomarkers of Exposure. No biomarkers of exposure to BDCM are currently known. By analogy with CCl_4, it is possible that BDCM may be metabolized to reactive intermediates that form covalent adducts with cellular macromolecules. If so, immunoassays and 32 P-post labeling assays might be capable of identifying and quantifying these adducts, although levels would likely be very low. Efforts to identify such adducts and to develop appropriate measurement techniques would be valuable for determining exposure.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. BDCM can be analyzed in water, air, and waste samples with good selectivity and sensitivity. However, since BDCM may be carcinogenic in humans, very low levels in water, air or other media may be of concern, so improvements in sensitivity would be valuable.

6.3.2 On-going Studies

The development of supercritical fluid (SCF) extraction holds great promise for analysis of nonpolar organic analytes such as BDCM. Current research in this area has been summarized by Hawthorne (1988). Research is ongoing to develop a "Master Analytical Scheme" for organic compounds in water (Michael et al. 1988), which includes BDCM as an analyte. The overall goal is to detect and quantify organic compounds at 0.1 μg/L (1 ppb)in drinking water, 1 μg /L in surface waters, and 10 μg/L in effluent waters. A comprehensive review of the literature leading up to these efforts has been published (Pellizzari et al. 1985).

The introduction of capillary column chromatography has markedly improved both sensitivity and resolution of gas chromatographic analysis, but because of the very small quantities of sample required, has made sample delivery more difficult. One of the more promising approaches to sample introduction using capillary columns with purgeand-trap collection is the use of cryofocusing. Basically, this procedure consists of collecting purged analyte on a short section of the capillary column cooled to a low temperature (e.g., -l00°C), followed by heating and backflushing of the sample onto the analytical column. Several compounds closely related to BDCM have been determined in water by this method (Washall and Wampler 1988), including mefhylene chloride, chloroform, chlorobromomethane and bromoform.

6. ANALYTICAL METHODS

Methods are also being developed for in situ measurement of organohalide levels in water. This has been demonstrated for chloroform-contaminated well water using remote fiber fluorimetry (RFF) and fiber optic chemical sensors (FOGS) (Milanovich 1986). With this approach, fluorescence of basic pyridine in the presence of organohalide (the Fujiwara reaction) is measured from a chemical sensor immersed in the water at the end of an optical fiber. If conditions can be found under which BDCM undergoes a Fujiwara reaction, its determination might be amendable to this approach.

The Environmental Health Laboratory Sciences Division of the Center for Environmental Health and Injury Control, Centers for Disease Control, is developing methods for the analysis of BDCM and other volatile organic compounds in blood. These methods use purge and trap methodology and magnetic mass spectrometry which gives detection limits in the low parts per trillion range.

7. REGULATIONS AND ADVISORIES

Because of its potential to cause adverse effects in exposed people, a number of regulations and advisory values have been established for BDCM by various national and state agencies. These values are summarized in Table 7-1. No international values were located.

7. REGULATIONS AND ADVISORIES

TABLE 7-1. Regulations and Guidelines Applicable to BDCM

Agency	Description	Value	References
	National		
Regulations			
a. Water			
EPA ODW	Maximum Contaminant Level (MCL) for Total Trihalomethanes	0.10 mg/L	40 CFR 141.12
	Monitoring Required for All Systems	NA[a]	40 CFR 141.40 EPA 1987a
EPA OSW	Groundwater Monitoring List (Appendix IX)	NA	40 CFR 264 EPA 1987b
EPA OWRS	General Permits Under the National Pollutant Discharge Elimination System (NPDES)	NA	40 CFR 122 Appendix D Table II
	General Pretreatment Regulations for Existing and New Sources of Pollution (halomethanes)	NA	40 CFR 403
FDA	Permissible Level in Bottled Water (Total Trihalomethanes)	0.10 mg/L	21 CFR 103.35
b. Non-specific Media			
EPA OERR	Reportable Quantity	5000 lb	40 CFR 302.4 EPA 1985a
Guidelines			
EPA OWRS	Ambient Water Quality Criteria to Protect Human Health[b]		EPA 1980b
	Ingesting Water and Organisms		
	10^{-5}	1.9 µg/L	
	10^{-6}	0.19 µg/L	
	10^{-7}	0.019 µg/L	
	Ingesting Organisms Only		
	10^{-5}	157 µg/L	
	10^{-6}	15.7 µg/L	
	10^{-7}	1.57 µg/L	
EPA	Reference Dose (RfD)	2E-2 mg/kg/d	EPA 1988
	State Regulations and Guidelines		
State Environmental Agencies	Drinking Water Standards and Guidelines		FSTRAC 1988
	Illinois	1.0 µg/L	
	Vermont	100 µg/L	

[a]Not applicable.

[b]Because of its carcinogenic potential, the EPA-recommended concentration for BDCM in ambient water is zero. However, because attainment of this level may not be possible, levels which correspond to upper bound incremental lifetime cancer risks of 10^{-5}, 10^{-6} and 10^{-7} are estimated. Since no quantitative data are available on the cancer risk from BDCM, the values are assumed to be equal to those for chloroform.

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9. GLOSSARY

Acute Exposure -- Exposure to a chemical for a duration of 14 days or less, as specified in the Toxicological Profiles.

Adsorption Coefficient (K_{oc}) -- The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

Adsorption Ratio (K_d) -- The amount of a chemical adsorbed by a sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

Bioconcentration Factor (BCF) -- The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same time period.

Cancer Effect Level (CEL) -- The lowest dose of chemical in a study or group of studies which produces significant increases in incidence of cancer (or tumors) between the exposed population and its appropriate control.

Carcinogen -- A chemical capable of inducing cancer.

Ceiling value (CL) -- A concentration of a substance that should not be exceeded, even instantaneously.

Chronic Exposure -- Exposure to a chemical for 365 days or more, as specified in the Toxicological Profiles.

Developmental Toxicity -- The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

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Embryotoxicity and Fetotoxicity -- Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the insult occurred. The terms, as used here, include malformations and variations, altered growth, and in utero death.

EPA Health Advisory -- An estimate of acceptable drinking water levels for a chemical substance based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

Immediately Dangerous to Life or Health (IDLH) -- The maximum environmental concentration of a contaminant from which one could escape within 30 min without any escape-impairing symptoms or irreversible health effects.

Intermediate Exposure -- Exposure to a chemical for a duration of 15-364 days, as specified in the Toxicological Profiles.

Immunologic Toxicity -- The occurrence of adverse effects on the immune system that may result from exposure to environmental agents such as chemicals.

In vitro -- Isolated from the living organism and artificially maintained, as in a test tube.

In vivo -- Occurring within the living organism.

Lethal Concentration (LO) (LC_{LO}) -- The lowest concentration of a chemical in air which has been reported to have caused death in humans or animals.

Lethal Concentration (50) (LC_{50}) -- A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose(LO) (LD_{LO}) -- The lowest dose of a chemical introduced by a route other than inhalation that is expected to have caused death in humans or animals.

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Lethal Dose (50) (LD_{50}) -- The dose of a chemical which has been calculated to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL) -- The lowest dose of chemical in a study or group of studies which produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

LT50 (lethal time) -- A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population,

Malformations -- Permanent structural changes that may adversely affect survival, development, or function.

Minimal Risk Level -- An estimate of daily human exposure to a chemical that is likely to be without an appreciable risk of deleterious effects (noncancerous) over a specified duration of exposure.

Mutagen -- A substance that causes mutations. A mutation is a change in the genetic material in a body cell. Mutations can lead to birth defects, miscarriages, or cancer.

Neurotoxicity -- The occurrence of adverse effects on the nervous system following exposure to a chemical.

No-Observed-Adverse-Effect Level (NOAEL) -- That dose of chemical at which there are no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Effects may be produced at this dose, but they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{ow}) -- The equilibrium ratio of the concentrations of a chemical in n-octanol and water, in dilute solution.

Permissible Exposure Limit (PEL) -- An allowable exposure level in workplace air averaged over an 8-h shift,

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q_1* -- The upper-bound estimate of the low-dose slope of the doseresponse curve as determined by the multistage procedure. The q_1* can be used to calculate an estimate of carcinogenic potency, the incremental excess cancer risk per unit of exposure (usually μg/L for water, mg/kg/day for food, and μg/m^3 for air).

Reference Dose (RfD) -- An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure of the human population to a potential hazard that is likely to be without risk of deleterious effects during a lifetime. The RfD is operationally derived from the NOAEL (from animal and human studies) by a consistent application of uncertainty factors that reflect various types of data used to estimate RfDs and an additional modifying factor, which is based on a professional judgment of the entire database on the chemical. The RfDs are not applicable to nonthreshold effects such as cancer.

Reportable Quantity (RQ) -- The quantity of a hazardous substance that is considered reportable under CERCLA. Reportable quantities are: (1) 1 lb or greater or (2) for selected substances, an amount established by regulation either under CERCLA or under Sect. 311 of the Clean Water Act. Quantities are measured over a 24-h period.

Reproductive Toxicity -- The occurrence of adverse effects on the reproductive system that may result from exposure to a chemical. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, 'or modifications in other functions that are dependent on the integrity of this system.

Short-Term Exposure Limit (STEL) -- The maximum concentration to which workers can be exposed for up to 15 min continually. No more than four excursions are allowed per day, and there must be at least 60 min between exposure periods. The daily TLV-TWA may not be exceeded.

Target Organ Toxicity -- This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

TD50 (toxic dose) -- A calculated dose of a chemical, introduced by a route other than inhalation, which is expected to cause a specific toxic effect in 50% of a defined experimental animal population.

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Teratogen -- A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV) -- A concentration of a substance to which most workers can be exposed without adverse effect. The TLV may be expressed as a TWA, as a STEL, or as a CL.

Time-weighted Average (TWA) -- An allowable exposure concentration averaged over a normal 8-h workday or 40-h workweek.

Uncertainty Factor (UF) -- A factor used in operationally deriving the RfD from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of humans, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using LOAEL data rather than NOAEL data. Usually each of these factors is set equal to 10.

APPENDIX: PEER REVIEW

A peer review panel was assembled for BDCM. The panel consisted of the following members: Dr. Sheldon Murphy, Professor of Environmental Health, University of Washington; Dr. Joseph Gould, Research Scientist, Georgia Institute of Technology; Dr. Nancy Reiches, Director of Research, Riverside Methodist Hospital, Columbus, Ohio; and Dr. Sanford Bigelow, President, MultiSciences, Inc. These experts collectively have knowledge of BDCM's physical and chemical properties, toxicokinetics, key health end points, mechanisms of action, human and animal exposure, and quantification of risk to humans. All reviewers were selected in conformity with the conditions for peer review specified in the Superfund Amendments and Reauthorization Act of 1986, Section 110.

A joint panel of scientists from ATSDR and EPA has reviewed the peer reviewers' comments and determined which comments will be included in the profile. A listing of the peer reviewers' comments not incorporated in the profile, with a brief explanation of the rationale for their exclusion, exists as part of the administrative record for this compound. A list of databases reviewed and a list of unpublished documents cited are also included in the administrative record.

The citation of the peer review panel should not be understood to imply their approval of the profile's final content. The responsibility for the content of this profile lies with the Agency for Toxic Substances and Disease Registry.

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TOXICOLOGICAL PROFILE FOR BROMOFORM AND DIBROMOCHLOROMETHANE

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

August 2005

DISCLAIMER

The use of company or product name(s) is for identification only and does not imply endorsement by the Agency for Toxic Substances and Disease Registry.

UPDATE STATEMENT

A Toxicological Profile for Bromoform and Dibromochloromethane, Draft for Public Comment was released in September 2003. This edition supersedes any previously released draft or final profile.

Toxicological profiles are revised and republished as necessary. For information regarding the update status of previously released profiles, contact ATSDR at:

Agency for Toxic Substances and Disease Registry
Division of Toxicology/Toxicology Information Branch
1600 Clifton Road NE
Mailstop F-32
Atlanta, Georgia 30333

FOREWORD

This toxicological profile is prepared in accordance with guidelines* developed by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA). The original guidelines were published in the *Federal Register* on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for the hazardous substance described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a hazardous substance's toxicologic properties. Other pertinent literature is also presented, but is described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

The focus of the profiles is on health and toxicologic information; therefore, each toxicological profile begins with a public health statement that describes, in nontechnical language, a substance's relevant toxicological properties. Following the public health statement is information concerning levels of significant human exposure and, where known, significant health effects. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to protection of public health are identified by ATSDR and EPA.

Each profile includes the following:

(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a hazardous substance to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects;

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure that present a significant risk to human health of acute, subacute, and chronic health effects; and

(C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

The principal audiences for the toxicological profiles are health professionals at the Federal, State, and local levels; interested private sector organizations and groups; and members of the public.

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed. Staff of the Centers for Disease Control and Prevention and other Federal scientists have also reviewed the profile. In addition, this profile has been peer-reviewed by a nongovernmental panel and was made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

Julie Louise Gerberding, M.D., M.P.H.
Administrator
Agency for Toxic Substances and
Disease Registry

*Legislative Background

The toxicological profiles are developed in response to the Superfund Amendments and Reauthorization Act (SARA) of 1986 (Public Law 99-499) which amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). This public law directed ATSDR to prepare toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. The availability of the revised priority list of 275 hazardous substances was announced in the *Federal Register* on November 7, 2003 (68 FR 63098) . For prior versions of the list of substances, see *Federal Register* notices dated April 17, 1987 (52 FR 12866); October 20, 1988 (53 FR 41280); October 26, 1989 (54 FR 43619); October 17,1990 (55 FR 42067); October 17, 1991 (56 FR 52166); October 28, 1992 (57 FR 48801); February 28, 1994 (59 FR 9486); April 29, 1996 (61 FR 18744); November 17, 1997 (62 FR 61332); October 21, 1999(64 FR 56792) and October 25, 2001 (66 FR 54014). Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list.

QUICK REFERENCE FOR HEALTH CARE PROVIDERS

Toxicological Profiles are a unique compilation of toxicological information on a given hazardous substance. Each profile reflects a comprehensive and extensive evaluation, summary, and interpretation of available toxicologic and epidemiologic information on a substance. Health care providers treating patients potentially exposed to hazardous substances will find the following information helpful for fast answers to often-asked questions.

Primary Chapters/Sections of Interest

Chapter 1: Public Health Statement: The Public Health Statement can be a useful tool for educating patients about possible exposure to a hazardous substance. It explains a substance's relevant toxicologic properties in a nontechnical, question-and-answer format, and it includes a review of the general health effects observed following exposure.

Chapter 2: Relevance to Public Health: The Relevance to Public Health Section evaluates, interprets, and assesses the significance of toxicity data to human health.

Chapter 3: Health Effects: Specific health effects of a given hazardous compound are reported by type of health effect (death, systemic, immunologic, reproductive), by route of exposure, and by length of exposure (acute, intermediate, and chronic). In addition, both human and animal studies are reported in this section.
NOTE: Not all health effects reported in this section are necessarily observed in the clinical setting. Please refer to the Public Health Statement to identify general health effects observed following exposure.

Pediatrics: Four new sections have been added to each Toxicological Profile to address child health issues:

Section 1.6 How Can (Chemical X) Affect Children?
Section 1.7 How Can Families Reduce the Risk of Exposure to (Chemical X)?
Section 3.7 Children's Susceptibility
Section 6.6 Exposures of Children

Other Sections of Interest:

Section 3.8 Biomarkers of Exposure and Effect
Section 3.11 Methods for Reducing Toxic Effects

ATSDR Information Center

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E-mail: atsdric@cdc.gov ***Internet***: http://www.atsdr.cdc.gov

The following additional material can be ordered through the ATSDR Information Center:

Case Studies in Environmental Medicine: Taking an Exposure History—The importance of taking an exposure history and how to conduct one are described, and an example of a thorough exposure history is provided. Other case studies of interest include *Reproductive and Developmental*

Hazards; Skin Lesions and Environmental Exposures; Cholinesterase-Inhibiting Pesticide Toxicity; and numerous chemical-specific case studies.

Managing Hazardous Materials Incidents is a three-volume set of recommendations for on-scene (prehospital) and hospital medical management of patients exposed during a hazardous materials incident. Volumes I and II are planning guides to assist first responders and hospital emergency department personnel in planning for incidents that involve hazardous materials. Volume III—*Medical Management Guidelines for Acute Chemical Exposures*—is a guide for health care professionals treating patients exposed to hazardous materials.

Fact Sheets (ToxFAQs) provide answers to frequently asked questions about toxic substances.

Other Agencies and Organizations

The National Center for Environmental Health (NCEH) focuses on preventing or controlling disease, injury, and disability related to the interactions between people and their environment outside the workplace. Contact: NCEH, Mailstop F-29, 4770 Buford Highway, NE, Atlanta, GA 30341-3724 • Phone: 770-488-7000 • FAX: 770-488-7015.

The National Institute for Occupational Safety and Health (NIOSH) conducts research on occupational diseases and injuries, responds to requests for assistance by investigating problems of health and safety in the workplace, recommends standards to the Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA), and trains professionals in occupational safety and health. Contact: NIOSH, 200 Independence Avenue, SW, Washington, DC 20201 • Phone: 800-356-4674 or NIOSH Technical Information Branch, Robert A. Taft Laboratory, Mailstop C-19, 4676 Columbia Parkway, Cincinnati, OH 45226-1998 • Phone: 800-35-NIOSH.

The National Institute of Environmental Health Sciences (NIEHS) is the principal federal agency for biomedical research on the effects of chemical, physical, and biologic environmental agents on human health and well-being. Contact: NIEHS, PO Box 12233, 104 T.W. Alexander Drive, Research Triangle Park, NC 27709 • Phone: 919-541-3212.

Referrals

The Association of Occupational and Environmental Clinics (AOEC) has developed a network of clinics in the United States to provide expertise in occupational and environmental issues. Contact: AOEC, 1010 Vermont Avenue, NW, #513, Washington, DC 20005 • Phone: 202-347-4976 • FAX: 202-347-4950 • e-mail: AOEC@AOEC.ORG • Web Page: http://www.aoec.org/.

The American College of Occupational and Environmental Medicine (ACOEM) is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. Contact: ACOEM, 25 Northwest Point Boulevard, Suite 700, Elk Grove Village, IL 60007-1030 • Phone: 847-818-1800 • FAX: 847-818-9266.

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THE PROFILE HAS UNDERGONE THE FOLLOWING ATSDR INTERNAL REVIEWS:

1. Health Effects Review. The Health Effects Review Committee examines the health effects chapter of each profile for consistency and accuracy in interpreting health effects and classifying end points.

2. Minimal Risk Level Review. The Minimal Risk Level Workgroup considers issues relevant to substance-specific Minimal Risk Levels (MRLs), reviews the health effects database of each profile, and makes recommendations for derivation of MRLs.

3. Data Needs Review. The Research Implementation Branch reviews data needs sections to assure consistency across profiles and adherence to instructions in the Guidance.

4. Green Border Review. Green Border review assures the consistency with ATSDR policy.

PEER REVIEW

A peer review panel was assembled for bromoform and dibromochloromethane. The panel consisted of the following members:

1. Bruce Jarnot, Ph.D., DABT, Senior Toxicologist, American Petroleum Institute, Washington DC;

2. Kannan Krishnan, Ph.D., Professor, Human Toxicology Research Group, University of Montreal Montreal Canada; and

3. Clint Skinner, Ph.D., Consultant, Skinner Associates, Creston California

These experts collectively have knowledge of bromoform and dibromochloromethane's physical and chemical properties, toxicokinetics, key health end points, mechanisms of action, human and animal exposure, and quantification of risk to humans. All reviewers were selected in conformity with the conditions for peer review specified in Section 104(I)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

Scientists from the Agency for Toxic Substances and Disease Registry (ATSDR) have reviewed the peer reviewers' comments and determined which comments will be included in the profile. A listing of the peer reviewers' comments not incorporated in the profile, with a brief explanation of the rationale for their exclusion, exists as part of the administrative record for this compound.

The citation of the peer review panel should not be understood to imply its approval of the profile's final content. The responsibility for the content of this profile lies with the ATSDR.

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1. PUBLIC HEALTH STATEMENT

This public health statement tells you about bromoform and dibromochloromethane and the effects of exposure to them.

The Environmental Protection Agency (EPA) identifies the most serious hazardous waste sites in the nation. These sites are then placed on the National Priorities List (NPL) and are targeted for long-term federal clean-up activities. Bromoform and dibromochloromethane has been found in at least 140 and 174 of the 1,662 current or former NPL sites. Although the total number of NPL sites evaluated for these substances is not known, the possibility exists that the number of sites at which bromoform and dibromochloromethane is found may increase in the future as more sites are evaluated. This information is important because these sites may be sources of exposure and exposure to these substances may harm you.

When a substance is released either from a large area, such as an industrial plant, or from a container, such as a drum or bottle, it enters the environment. Such a release does not always lead to exposure. You can be exposed to a substance only when you come in contact with it. You may be exposed by breathing, eating, or drinking the substance, or by skin contact.

If you are exposed to bromoform or dichlorobromomethane, many factors will determine whether you will be harmed. These factors include the dose (how much), the duration (how long), and how you come in contact with them. You must also consider any other chemicals you are exposed to and your age, sex, diet, family traits, lifestyle, and state of health.

1.1 WHAT ARE BROMOFORM AND DIBROMOCHLOROMETHANE?

Bromoform (also known as tribromomethane) and dibromochloromethane are colorless to yellow, heavy, nonburnable liquids with a sweetish odor. These chemicals are possible contaminants of drinking water that has been chlorinated to kill bacteria and viruses that could cause serious waterborne infectious diseases. Bromoform and dibromochloromethane may form

when chlorine reacts with other naturally occurring substances in water, such as decomposing plant material. Plants in the ocean also produce small amounts of these chemicals.

These chemicals are found mainly in water that originally came from surface sources, such as rivers and lakes. Springs and deep drilled wells usually contain very little of the substances that react with chlorine to form these chemicals; therefore, well and spring water is less likely a source of bromoform and dibromochloromethane than water from a reservoir (artificial lake). The amount of bromoform and dibromochloromethane in drinking water can change considerably from day to day, depending on the source, temperature, amount of plant material in the water, amount of chlorine added, and a variety of other factors.

In the past, bromoform was used by industry to dissolve dirt and grease and to make other chemicals. It was also used in the early part of this century as a medicine to help children with whooping cough get to sleep. Currently, bromoform is only produced in small amounts for use in laboratories and in geological and electronics testing. Dibromochloromethane was used in the past to make other chemicals such as fire extinguisher fluids, spray can propellants, refrigerator fluid, and pesticides. It is now only used on a small scale in laboratories.

In the environment, bromoform and dibromochloromethane are not found as pure liquids, but instead, they are found either dissolved in water or evaporated into air as a gas. Both bromoform and dibromochloromethane are relatively stable in the air, but reactions with other chemicals in the air cause them to break down slowly (about 50% in 1 or 2 months). Bromoform and dibromochloromethane in water or soil may also be broken down by bacteria, but the speed of this process is not known.

Further information on the properties, uses, and chemical identity of bromoform and dibromochloromethane in the environment may be found in Chapters 4, 5, and 6.

1.2 WHAT HAPPENS TO BROMOFORM AND DIBROMOCHLOROMETHANE WHEN THEY ENTER THE ENVIRONMENT?

Bromoform and dibromochloromethane enter the environment through the disposal of water that has been disinfected with chlorine or as vapors emitted from chlorinated water. These chemicals are also made naturally by plant-like organisms called algae that are found in the oceans. Some part of bromoform and dibromochloromethane that enters the air is removed by rain. What is left in the air takes about 1–2 months for half of it to degrade. In water, bromoform and dibromochloromethane are slowly broken down at the water surface where oxygen is available, but break down much faster in deep water and in water that is underground where there is a lot less oxygen. Bromoform and dibromochloromethane are mobile in soils and may seep into groundwater. Bromoform and dibromochloromethane do not appear to concentrate in fish.

1.3 HOW MIGHT I BE EXPOSED TO BROMOFORM AND DIBROMOCHLORO-METHANE?

You are most likely to be exposed to bromoform and dibromochloromethane by drinking water that has been treated with chlorine. Usually, the levels in chlorinated drinking water are between 1 and 10 parts of bromoform and dibromochloromethane per billion parts of water (ppb). These are levels that are known to be without adverse health effects. Bromoform and dibromochloro-methane have also been detected in chlorinated swimming pools. Exposure can occur at a swimming pool, by breathing bromoform or dibromochloromethane that has evaporated into the air, or by uptake from the water through the skin. Neither dibromochloromethane nor bromoform are likely to be found in food.

If you live near a factory or laboratory that makes or uses dibromochloromethane or bromoform, you might be exposed to dibromochloromethane or bromoform in the air. Currently, bromoform is only used for geological and electronics testing. Dibromochloromethane is used on a small-scale in laboratories. Since neither dibromochloromethane nor bromoform have widespread use in this country, they are usually present in outside air at very low levels (less than 0.01 ppb). Therefore, exposure to bromoform or dibromochloromethane in the air is a minor route. Exposure may occur if you come into contact with water or soil at a chemical waste site where

dibromochloromethane or bromoform has been disposed. Further information on how you might be exposed to these chemicals is given in Chapter 6.

1.4 HOW CAN BROMOFORM AND DIBROMOCHLOROMETHANE ENTER AND LEAVE MY BODY?

Studies in animals or humans indicate that both bromoform and dibromochloromethane can easily enter your body after swallowing them in water or breathing them in air. They can also enter your body through your skin (for example, by washing or showering in water containing these chemicals). Some portion of bromoform and dibromochloromethane entering your body may be broken down to other compounds. Bromoform, dibromochloromethane, and their breakdown products can be removed from the body by being exhaled from the lungs. These chemicals leave the body fairly rapidly. Bromoform and dibromochlormethane do not tend to build up in the body, 50–90% of the amount that enters the body is removed within 8 hours. Further information on how bromoform and dibromochloromethane enter and leave your body is given in Chapter 3.

1.5 HOW CAN BROMOFORM AND DIBROMOCHLOROMETHANE AFFECT MY HEALTH?

Scientists use many tests to protect the public from harmful effects of toxic chemicals and to find ways for treating persons who have been harmed.

One way to learn whether a chemical will harm people is to determine how the body absorbs, uses, and releases the chemical. For some chemicals, animal testing may be necessary. Animal testing may also help identify health effects such as cancer or birth defects. Without laboratory animals, scientists would lose a basic method for getting information needed to make wise decisions that protect public health. Scientists have the responsibility to treat research animals with care and compassion. Scientists must comply with strict animal care guidelines because laws today protect the welfare of research animals.

1. PUBLIC HEALTH STATEMENT

The effects of bromoform and dibromochloromethane on your health depend largely on the amount you take into your body and the duration of exposure. In general, the more you take in, the greater the chance that an effect will occur. The main effect of swallowing or breathing large amounts of bromoform is a slowing of normal brain activities, resulting in sleepiness or sedation occuring quickly after the chemicals enter your body. In humans, these effects tend to disappear within a day. Exposures capable of producing these effects include swallowing 1–4 drops of liquid bromoform, an amount much greater than is usually found in a glass of drinking water. At much higher amounts, a person may become unconsciousness or die. The amount of dibromochloromethane taken by mouth that would affect humans is not known, but is probably similar to bromoform.

Some studies in animals indicate that exposure to high doses of bromoform or dibromochloromethane may also lead to liver and the kidney injury within a short period of time. Exposure to low levels of bromoform or dibromochloromethane do not appear to seriously affect the brain, liver, or kidneys. Other animal studies suggest that typical bromoform or dibromochloromethane exposures do not pose a high risk of affecting the chance of becoming pregnant or harming an unborn baby. However, studies in animals indicate that long-term intake of either bromoform or dibromochloromethane can cause liver and kidney cancer. Although cancer in humans cannot be definitely attributed to these chemicals, it is an effect of special concern, since many people are exposed to low levels of bromoform and dibromochloromethane in chlorinated drinking water.

The International Agency for Research on Cancer (IARC) concluded that bromoform and dibromochloromethane are not classifiable as to human carcinogenicity. The EPA classified bromoform as a probable human carcinogen and dibromochloromethane as a possible human carcinogen.

Further information on how bromoform and dibromochloromethane can affect the health of humans and animals is presented in Chapter 3.

1.6 HOW CAN BROMOFORM AND DIBROMOCHLOROMETHANE AFFECT CHILDREN?

This section discusses potential health effects in humans from exposures during the period from conception to maturity at 18 years of age.

In the early 1900s, bromoform was given to children suffering from whooping cough, resulting in several deaths when children were accidentally overdosed. Children appeared drowsy, then lifeless, just before dying.

There are no studies in humans or laboratory animals that examine whether the effects of bromoform and dibromochloromethane change with age. Based on current knowledge of body function, and metabolism in the body, there is no indication that children will be affected more than adults.

1.7 HOW CAN FAMILIES REDUCE THE RISK OF EXPOSURE TO BROMOFORM AND DIBROMOCHLOROMETHANE?

If your doctor finds that you have been exposed to substantial amounts of bromoform and/or dibromochloromethane, ask whether your children might also have been exposed. Your doctor might need to ask your state health department to investigate.

The chance of consuming bromoform or dibromochloromethane in chlorinated public drinking water varies with season, water temperature, water chemistry, disinfection method, and other factors. However, the health risks associated with drinking non-disinfected water when there is evidence of disease-causing contamination (i.e., bacteria, viruses, etc.) are much greater than the risk of adverse health effects from exposure to bromoform or dibromochloromethane.

There are water treatment methods that people can be used in the home to reduce exposure to bromoform and dibromochloromethane from chlorinated tap water. These include simple do-it-yourself methods such as connecting solid carbon black filters to faucets and shower taps. Homeowners may discuss other home water treatment methods, including filtering, aeration or

boiling, distillation, and/or activated charcoal, with a professional plumber or water well contractor. The chance of exposure to bromoform and dibromochloromethane may be reduced by minimizing contact with water expected to have higher levels of these chemicals, such as chlorinated swimming pool water. When bathing or showering some portion of dibromochloromethane and/or bromoform may evaporate into the air. Opening bathroom windows, and taking shorter baths and showers may reduce the amount of chemical vapor that is inhaled or absorbed through the skin.

1.8 IS THERE A MEDICAL TEST TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO BROMOFORM AND DIBROMOCHLOROMETHANE?

If you are exposed to bromoform or dibromochloromethane, measurable levels of the chemicals can sometimes be detected in samples of your blood, breath, or fat. However, there is not enough information at present to use the results of such tests to estimate the level of exposure or to predict the nature or the severity of any health effects that might result. Since special equipment is needed, these tests are not routinely performed in doctors' offices. Because bromoform and dibromochloromethane are quickly eliminated from the body, these special laboratory tests are only effective in detecting recent exposures (within 1 or 2 days). Further information on how bromoform and dibromochloromethane can be measured in exposed humans is presented in Chapters 3 and 7.

1.9 WHAT RECOMMENDATIONS HAS THE FEDERAL GOVERNMENT MADE TO PROTECT HUMAN HEALTH?

The federal government develops regulations and recommendations to protect public health. Regulations *can* be enforced by law. The EPA, the Occupational Safety and Health Administration (OSHA), and the Food and Drug Administration (FDA) are some federal agencies that develop regulations for toxic substances. Recommendations provide valuable guidelines to protect public health, but *cannot* be enforced by law. The Agency for Toxic Substances and Disease Registry (ATSDR) and the National Institute for Occupational Safety

and Health (NIOSH) are two federal organizations that develop recommendations for toxic substances.

Regulations and recommendations can be expressed as “not-to-exceed” levels, that is, levels of a toxic substance in air, water, soil, or food that do not exceed a critical value that is usually based on levels that affect animals; they are then adjusted to levels that will help protect humans. Sometimes these not-to-exceed levels differ among federal organizations because they used different exposure times (an 8-hour workday or a 24-hour day), different animal studies, or other factors.

Recommendations and regulations are also updated periodically as more information becomes available. For the most current information, check with the federal agency or organization that provides it. Some regulations and recommendations for bromoform and dibromochloromethane include the following:

OSHA has set a legally enforceable limit of 0.5 ppm for bromoform in workroom air to protect workers during an 8-hour shift over a 40-hour work week.

EPA recommends that drinking water levels for bromoform should not be more than 0.7 parts per million (ppm) for bromoform and 0.7 ppm for dibromochloromethane.

1.10 WHERE CAN I GET MORE INFORMATION?

If you have any more questions or concerns, please contact your community or state health or environmental quality department, or contact ATSDR at the address and phone number below.

ATSDR can also tell you the location of occupational and environmental health clinics. These clinics specialize in recognizing, evaluating, and treating illnesses that result from exposure to hazardous substances.

Toxicological profiles are also available on-line at www.atsdr.cdc.gov and on CD-ROM. You may request a copy of the ATSDR ToxProfilesTM CD-ROM by calling the toll-free information and technical assistance number at 1-888-42ATSDR (1-888-422-8737), by e-mail at atsdric@cdc.gov, or by writing to:

Agency for Toxic Substances and Disease Registry
Division of Toxicology
1600 Clifton Road NE
Mailstop F-32
Atlanta, GA 30333
Fax: 1-770-488-4178

Organizations for-profit may request copies of final Toxicological Profiles from the following:

National Technical Information Service (NTIS)
5285 Port Royal Road
Springfield, VA 22161
Phone: 1-800-553-6847 or 1-703-605-6000
Web site: http://www.ntis.gov/

2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO BROMOFORM AND DIBROMOCHLOROMETHANE IN THE UNITED STATES

Bromoform ($CHBr_3$; CAS Number 75-25-2), also known as tribromomethane, and dibromochloromethane ($CHClBr_2$; CAS Number 124-48-1) belong to a group of chemicals referred to as trihalomethanes; the other two chemicals in this group are chloroform (also known as trichloromethane) and dichlorobromomethane. Trihalomethanes are formed when raw source water is disinfected by chlorination. In the United States, over 280 million people are served by public water systems that apply chlorine or some of its compounds as disinfectants to water in order to provide protection against microbial contaminants that otherwise might cause serious water-borne diseases. While these chlorine-containing disinfectants are effective in controlling many microorganisms, they react with natural organic or carbon-containing matter in the water to form disinfection byproducts. Therefore, the principal source of human exposure to bromoform and dibromochloromethane is chlorinated water supplied to homes, work, and public places. Bromoform and dibromochloromethane concentrations in public supply or tap water are in the low microgram/L range. Dibromochloromethane is often found more frequently than bromoform in samples from chlorinated water systems.

In the past, it was thought that most of the human exposure to bromoform and dibromochloromethane occurred through consumption of chlorinated drinking water. However, because of their physical properties (see Henry's law constants in Chapter 4), some bromoform and dibromochloromethane volatilize into the air from normal household use of water containing these chemicals. Recent models for residential exposure predict that exposure by the inhalation and dermal routes may be significant. Dermal exposure is expected from showering or bathing. Total administered doses of bromoform or dibromochloromethane for residential tap water having low microgram/L concentrations are predicted to be on the order of 10^{-4} mg/kg/day.

2.2 SUMMARY OF HEALTH EFFECTS

The general population is primarily exposed to bromoform and dibromochloromethane via tap water. The primary routes of exposure are ingestion and inhalation (from volatized compounds) and dermal exposure during showering and bathing. Bromoform and dibromochloromethane are readily absorbed from the

gastrointestinal tract and may be absorbed through the respiratory tract and skin. They are rapidly distributed throughout the body. In the liver, bromoform and dibromochloromethane are metabolized by the cytochrome P-450 mixed function oxidase system to a highly reactive metabolite, which is ultimately metabolized to carbon dioxide or carbon monoxide.

Studies in animals, combined with limited observations in humans, indicate that the principal adverse health effects associated with inhalation or oral exposure to bromoform or dibromochloromethane are central nervous system depression and liver and kidney damage. Although limited dermal data were located, it is likely that similar adverse health effects would occur from dermal exposure. Based on the no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL) values identified in animal studies, the liver appears to be the most sensitive target organ. Two types of liver effects have been observed in laboratory animals: lipidosis and hepatocellular necrosis. Lipidosis is an accumulation of lipids in the hepatocytes resulting in cellular vacuolization and swelling. Hepatocellular necrosis is observed at higher doses. It is not known if these effects represent a continuum of liver damage or are due to separate modes of action. Kidney effects are typically observed at higher doses than the hepatic effects; tubular cell degeneration and nephrosis are the most commonly reported effects in laboratory animals. Central nervous system depression, as evidenced by lethargy, ataxia, and shallow breathing, is typically observed at very high, often lethal, doses. High-dose exposure is also associated with decreases in body weight gain. There are limited data on the immunotoxicity of bromoform and dibromochloromethane. Impaired humoral and cell-mediated (only observed with dibromochloromethane) immunity were observed in a study of mice administered bromoform or dibromochloromethane via gavage for 14 days. For bromoform, the immune and liver effects occurred at the same dose level; for dibromochloromethane, the immune effects occurred at a lower dose than liver effects.

The available data on the potential of bromoform and dibromochloromethane to induce reproductive and/or developmental effects are inconclusive. Human data primarily come from epidemiological studies of pregnancy outcomes in women exposed to trihalomethanes in drinking water. These studies involved mixed exposures to the trihalomethane compounds (chloroform, dichlorobromomethane, bromoform, and dibromochloromethane), and many did not analyze for possible risks associated with bromoform or dibromochloromethane water concentrations. These data are inadequate for establishing a causal relationship between trihalomethane exposure and reproductive and/or developmental toxicity. The animal data suggest that exposure to bromoform or dibromochloromethane does not cause histological damage to reproductive organs or impair reproductive function; although high-dose exposure may result in reduced fertility. The available developmental toxicity data suggest that bromoform and

dibromochloromethane may be toxic to the developing fetus, but these data are inadequate to establish firm conclusions.

The carcinogenicity of bromoform and dibromochloromethane has been studied in both humans and laboratory animals. The human data consist of studies of trihalomethane exposure via tap water. As with the reproductive and developmental toxicity studies, these data are inconclusive and do not establish causal relationships. Carcinogenic effects have been observed in animals exposed to bromoform and dibromochloromethane. Chronic oral exposure to bromoform resulted in increases in the occurrence of intestinal tumors in female rats. Dibromochloromethane induced liver tumors in male and female mice. The Department of Health and Human Services (DHHS) has not categorized the human carcinogenic potential of bromoform or dibromochloromethane. The International Agency for Research on Cancer (IARC) concluded that there were inadequate human data and limited animal data and assigned bromoform and dibromochloromethane to weight of evidence category 3, not classifiable as to carcinogenicity in humans. EPA classified bromoform as a probable human carcinogen, group B2 and dibromochloromethane as a possible human carcinogen, group C.

The primary targets of bromoform and dibromochloromethane toxicity—liver, kidney, and central nervous system—are discussed in greater detail below. The reader is referred to Section 3.2, Discussion of Health Effects by Route of Exposure, for additional information on other health effects.

Liver Effects. Acute, intermediate-, and chronic-duration studies in laboratory animals provide strong evidence that the liver is the critical target of bromoform and dibromochloromethane toxicity. There are very limited human data on the toxicity of these two compounds; data for other trihalomethanes, particularly chloroform, suggest that the liver would also be a target of toxicity in humans.

There is strong evidence that the hepatotoxicity of bromoform and dibromochloromethane is due to their metabolism to reactive intermediates and highly reactive trihalomethyl free radicals. At lower doses, the hepatotoxicity of bromoform and dibromochloromethane is characterized by fatty infiltration, cellular vacuolization and swelling, and increases in liver weight. Consistent with the accumulation of lipids is the observed decrease in serum triglyceride levels and alterations in serum cholesterol levels. At higher doses, focal centrolobular necrosis and increases in SGOT and SGPT levels have been observed. For bromoform, the LOAEL is 50 mg/kg (5 days/week; 36 mg/kg/day) for fatty changes observed in rats administered bromoform in corn oil via gavage for 13 weeks. Necrosis was observed in rats receiving gavage doses of 200 mg/kg (143 mg/kg/day), 5 days/week for 2 years, but not after 13 weeks of dosing.

For dibromochloromethane, the LOAEL is 40 mg/kg (5 days/week; 28 mg/kg/day) for fatty liver changes in rats receiving gavage doses of dibromochloromethane in corn oil for 2 years. Necrosis was observed at 173 mg/kg/day in rats exposed via the diet for 1 month.

Species and possible gender differences in the hepatotoxicity of bromoform and dibromochloromethane have been identified. Rats appear to be more sensitive than mice to the liver effects. Under similar exposures scenarios, respective NOAEL and LOAEL values of 25 and 50 mg/kg (5 days/week) for fatty changes were identified in rats exposed to bromoform for 13 weeks; the NOAEL and LOAEL values in mice were 100 and 200 mg/kg (5 days/week), respectively. For dibromochloromethane, the NOAEL and LOAEL values for fatty changes following a 13-week gavage exposure (5 days/week) were 30 and 60 mg/kg for rats and 125 and 250 mg/kg for mice.

Kidney Effects. Renal effects have not been consistently found in studies of laboratory animals, particularly in the case of bromoform exposure. One study reported mesangial nephrosis in mice exposed to 145 mg/kg/day bromoform via gavage for 14 days and identified a NOAEL of 37 mg/kg/day. Comprehensive intermediate- and chronic-duration studies in rats and mice did not find significant renal effects at doses as high as 400 mg/kg (5 days/week; 286 mg/kg/day). In contrast, exposure to dibromochloromethane resulted in mesangial hyperplasia in mice exposed to ≥37 mg/kg/day via gavage for 14 days. Toxic nephropathy was observed in rats and mice exposed to 250 mg/kg (179 mg/kg/day) for 13 weeks and in mice receiving gavage doses of 100 mg/kg (71 mg/kg/day) for 2 years.

Central Nervous System Depression. In children, oral doses of around 60 mg/kg/day of bromoform typically produced only mild sleepiness, while doses of 150 mg/kg sometimes produced stupor or deep narcosis, usually accompanied by depressed respiration and erratic heartbeat. The onset of sedation after ingestion is rapid, reportedly minutes in children and about an hour in mice. In intermediate and chronic oral studies in animals, doses of bromoform ≥100 mg/kg (5 days/week; 71 mg/kg/day) caused lethargy. Airborne concentrations of bromoform leading to central nervous system depression in humans are not known, but brief exposures of laboratory animals to high concentrations (7,000 ppm) leads to deep sedation within minutes. Central nervous system effects were also observed in laboratory animals at a concentration of 240 ppm in a short-term, repeated dose study. These depressant effects on the nervous system appear to be fully reversible both in animals and humans, but it is difficult to rule out the possibility of subtle, but enduring, neurological changes following narcotizing doses.

2.3 MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for bromoform and dibromochloromethane. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

The details regarding calculations of the MRLs for bromoform and dibromochloromethane are described in Appendix A.

Inhalation MRLs

MRLs for acute- (≤14 days), intermediate- (15–364 days), and chronic-duration (≥364 days) inhalation exposure to bromoform or dibromochloromethane have not been derived because quantitative data were not available to determine NOAELs or LOAELs.

Information on the toxicity of bromoform or dibromochloromethane in humans following inhalation exposure was not available. Brief summaries of adverse effects in laboratory animals following inhalation exposure to bromoform, reported in abstract form, do not provide sufficient basis for MRL derivation. No studies were located regarding effects of dibromochloromethane in animals exposed via inhalation. Therefore, inhalation MRLs were not derived for either bromoform or dibromochloromethane.

Oral MRLs

Bromoform

- An MRL of 0.7 mg/kg/day has been derived for acute-duration oral exposure (14 days or less) to bromoform.

The acute toxicity of bromoform has been investigated in a number of animal studies. These studies have identified several targets of toxicity. The available data suggest that the liver is the most sensitive target. The threshold for liver effects appears to be between 50 and 125 mg/kg/day. Increases in absolute and relative liver weights were observed in mice exposed to 125 mg/kg/day bromoform in emulphor in water for 14 days (Munson et al. 1982). Centrilobular pallor, considered to be indicative of hepatocellular degeneration, was observed at 145 mg/kg/day in mice receiving gavage doses of bromoform in corn oil for 14 days (Condie et al. 1983). Hepatocellular vacuolization and/or swelling was observed at 200 mg/kg (9 days/11 days) and higher (Coffin et al. 2000). Other effects observed in acute-duration animal studies include mesangial nephrosis in mice exposed to 145 mg/kg/day via gavage for 14 days (Condie et al. 1983), central nervous system depression, as evidenced by lethargy, labored and shallow breathing, and ataxia in rats and mice at ≥600 mg/kg/day for 1–14 days (Balster and Borzelleca 1982; Bowman et al. 1978; NTP 1989a), and developmental effects (increases in the occurrence of skeletal anomalies) in the offspring of rats exposed to 200 mg/kg/day on gestational days 6–15 (Ruddick et al. 1983).

The Condie et al. (1983) and Munson et al. (1982) studies identify the lowest LOAELs for liver effects. The Condie et al. (1983) study identified a NOAEL of 72 mg/kg/day and LOAEL of 145 mg/kg/day for centrilobular pallor in mice receiving daily gavage doses of bromoform in corn oil for 14 days. Focal inflammation and increase in SGPT levels were observed at 280 mg/kg/day. The Munson et al. (1982) study identified a NOAEL of 50 mg/kg/day and LOAEL of 125 mg/kg/day for increases in absolute and relative liver weights in mice receiving daily gavage doses of bromoform in a 10% emulphor/de-ionized water solution for 14 days. At 250 mg/kg/day, increases in SGOT and SGPT levels were also observed. The Condie et al. (1983) study was selected as the basis of the MRL because it identified a higher NOAEL for liver effects than the Munson et al. (1982) study and included histopathological examination of the liver which was not included in the Munson et al. (1982) study. The NOAEL of 72 mg/kg/day was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) to yield an acute-duration oral MRL of 0.7 mg/kg/day.

- An MRL of 0.2 mg/kg/day has been derived for intermediate-duration oral exposure (15–364 days) to bromoform.

The oral toxicity database in animals provides strong evidence that the liver is the most sensitive target of bromoform toxicity. Several intermediate-duration studies have reported liver effects, typically at the lowest dose level. At lower doses, fatty changes, characterized as hepatocellular vacuolization and swelling, were observed in rats and mice. Focal necrosis was observed at higher oral doses. The lowest LOAEL for liver effects identified in an intermediate-duration study is 50 mg/kg in rats receiving gavage doses of bromoform in corn oil 5 days/week for 13 weeks (NTP 1989a); this study identified a NOAEL of 25 mg/kg. The intermediate-duration oral MRL of 0.2 mg/kg/day for bromoform was derived by applying an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) to the duration-adjusted NOAEL of 18 mg/kg/day.

- An MRL of 0.02 mg/kg/day has been derived for chronic-duration oral exposure (365 days or more) to bromoform.

Three studies have examined the chronic toxicity of bromoform in animals. Rat and mouse studies conducted by NTP (1989a) are comprehensive studies that found fatty liver changes (hepatocellular vacuolization) at the lowest dose tested, 100 mg/kg (5 days/week; 71 mg/kg/day). The third study (Tobe et al. 1982) identified a similar LOAEL (140 mg/kg/day) in rats exposed to bromoform in the diet for 2 years; this study also identified a NOAEL of 35 mg/kg/day. At 140 mg/kg/day, yellowing of the liver and increased absolute and relative liver weights were observed. The NTP (1989a) rat study was selected as the basis of the chronic-duration oral MRL for bromoform. Even though the Tobe et al. (1982) study identified a NOAEL for liver effects, this study was not selected as the critical study because no histological examination of the liver was conducted and the results were poorly reported. The duration-adjusted LOAEL of 71 mg/kg/day was divided by an uncertainty factor of 300 (3 for use of a minimal LOAEL, 10 for animal to human extrapolation, and 10 for human variability) and a modifying factor of 10 to account for the identification of a lower LOAEL in a 13-week study (NTP 1989a) resulting in an MRL of 0.02 mg/kg/day.

Dibromochloromethane

- An MRL of 0.1 mg/kg/day has been derived for acute-duration oral exposure (14 days or less) to dibromochloromethane.

The available data on the oral toxicity of dibromochloromethane in animals strongly suggest that the liver is the most sensitive target of toxicity. In most studies, liver effects are observed at lower doses than kidney effects (the next most sensitive end point). The study of Condie et al. (1983) was selected as the basis for the acute-duration oral MRL for dibromochloromethane because it showed dose-related incidences of liver and kidney lesions and identified the lowest LOAEL for liver effects among the available studies. A LOAEL of 37 mg/kg/day, the lowest dose tested, was identified for liver damage (hepatocellular vacuolization) in mice administered dibromochloromethane in corn oil for 14 consecutive days. A reliable NOAEL for liver or kidney effects could not be determined from the available acute data. Applying an uncertainty factor of 300 (3 for use of a minimal LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability) to the LOAEL of 37 mg/kg/day yields an acute-duration oral MRL of 0.1 mg/kg/day for dibromochloromethane.

No data were located regarding the toxicity of dibromochloromethane following intermediate-duration oral exposure in humans. A number of intermediate-duration studies of rats and mice were located. The liver was identified as the most sensitive target. Hepatocellular vacuolization was observed at 43 mg/kg/day (60 mg/kg, 5 days/week) and higher (Aida et al. 1992; Daniel et al. 1990; NTP 1985); the highest NOAEL for liver effects is 21 mg/kg/day (30 mg/kg, 5 days/week) (NTP 1985). At higher doses (≥100 mg/kg/day), proximal tubular degeneration and nephropathy were observed (Daniel et al. 1990; NTP 1985). Impaired humoral immune function was observed in mice administered 125 mg/kg/day via gavage for 14 days (Munson et al. 1982). Several animal studies also reported neurological effects: decreases in brain weight and decreases in operant behavior at ≥100 mg/kg/day (Balster and Borzelleca 1982; Daniel et al. 1990). Borzelleca and Carchman (1982) found decreases in fertility at high dibromomochloromethane doses (685 mg/kg/day).

Derivation of an intermediate-duration oral MRL for dibromochloromethane based on the NTP (1985) rat study, which identified NOAEL and LOAEL values of 21 and 43 mg/kg/day, was considered. However, the resultant MRL would be higher than the acute-duration oral MRL.

- An MRL of 0.09 mg/kg/day has been derived for chronic-duration oral exposure (365 days or less) to dibromochloromethane.

Studies of dibromochloromethane consistently indicate that the liver is a target organ. The NTP (1985) study, in which rats received gavage doses of 0, 40, or 80 mg/kg of dibromochloromethane in corn oil, 5 days/week, for 104 weeks, was selected as the basis for the chronic-duration oral MRL. This study (NTP 1985) was selected because it showed dose-related incidences of microscopic hepatic lesions and

also identified the lowest LOAEL of 40 mg/kg (duration-adjusted LOAEL of 28 mg/kg/day) for hepatic effects in chronic studies (NTP 1985; Tobe et al. 1982) of dibromochloromethane toxicity. A chronic-duration oral MRL of 0.09 mg/kg/day was derived by applying an uncertainty factor of 300 (3 for use of a minimal LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability) to the LOAEL of 28 mg/kg/day.

3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of bromoform and dibromochloromethane. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between

"less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of bromoform or dibromochloromethane are indicated in Tables 3-1 and 3-2 and Figures 3-1 and 3-2. Because cancer effects could occur at lower exposure levels, Figures 3-1 and 3-2 also shows a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

3.2.1 Inhalation Exposure

No studies were located regarding health effects of bromoform or dibromochloromethane in humans following inhalation exposure. In animals, there are limited data from several older studies on the effects of inhalation exposure to bromoform; no studies were located regarding effects of dibromochloro-methane.

3.2.1.1 Death

Inhalation of very high concentrations (56,000 or 84,000 ppm) of bromoform vapor for 1 hour has been reported to cause death in dogs (Merzbach 1928). The chief symptoms noted were initial excitation

followed by deep sedation. This indicates that central nervous system depression is probably the chief cause of death in such acute exposures. Because only two animals were used (one animal per dose) and only high doses were administered, these data do not provide a reliable estimate of the minimum lethal concentration in dogs or other animal species.

3.2.1.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, endocrine, dermal, ocular, or body weight effects in animals or humans following inhalation exposure to bromoform or dibromochloromethane.

Hepatic Effects. Two studies (Dykan 1962, 1964; published in Russian and available only as the English abstract) indicate that inhalation exposure of animals to high concentrations of bromoform leads to hepatic injury. Exposure of rats to 240 ppm of bromoform for 10 days resulted in dystrophic and vascular changes in the liver, with altered hepatic metabolism (Dykan 1964). Longer-term exposure (2 months) to concentrations of 24 ppm also led to hepatic changes (decreased blood clotting and impaired glycogenesis) (Dykan 1962). No significant alterations were observed after exposure to 4.8 ppm (Dykan 1964). These changes appear to resemble the changes produced after oral exposure to bromoform (see Section 3.2.2.2), suggesting that the hepatotoxicity of bromoform is not route-specific.

Renal Effects. Similar to the hepatic effects, exposure to 240 ppm bromoform for 10 days resulted in dystrophic and vascular changes in the kidney with altered renal filtration (Dykan 1964). A 2-month exposure to 24 ppm resulted in proteinuria and decreased creatinine clearance (Dykan 1962). A concentration of 4.8 ppm was estimated to be without significant effects on the kidney (Dykan 1964). These changes appear to resemble the changes produced after oral exposure to bromoform (see Section 3.2.2.2).

3.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans or animals after inhalation exposure to bromoform or dibromochloromethane.

3.2.1.4 Neurological Effects

Inhalation exposure to high levels (29,000 ppm or above) of bromoform has been observed to lead to rapid and profound depression of the central nervous system in dogs (Graham 1915; Merzbach 1928). This is presumably due to a nonspecific anesthetic effect similar to that produced by various other volatile halocarbons. Obvious clinical signs included deep relaxation and sedation (Merzbach 1928). Clinical signs of nervous system depression appeared quickly (within minutes), and tended to disappear within a day after exposure ceased (Graham 1915).

No studies were located regarding the following effects in humans or animals after inhalation exposure to bromoform or dibromochloromethane:

3.2.1.5 Reproductive Effects

3.2.1.6 Developmental Effects

3.2.1.7 Cancer

3.2.2 Oral Exposure

Most information on the health effects of bromoform and dibromochloromethane comes from studies in animals (rats and mice) exposed by the oral route. For bromoform, there are some observations in humans stemming from the past use of bromoform as a sedative, but no studies were located on the effect of dibromochloromethane in humans. Summaries of studies that provide reliable quantitative toxicity data are presented in Table 3-1 and Figure 3-1 for bromoform and in Table 3-2 and Figure 3-2 for dibromochloromethane. The main conclusions from these studies are discussed below.

3.2.2.1 Death

Bromoform. In the early part of this century, bromoform was often given as a sedative to children suffering from whooping cough, and several deaths due to accidental overdoses have been described (Dwelle 1903; Kobert 1906; Roth 1904 as cited in von Oettingen 1955). The principal clinical signs in fatal cases were those of severe central nervous system depression (unconsciousness, stupor, and loss of reflexes), and death was generally the result of respiratory failure (von Oettingen 1955). If death could be averted, recovery was generally complete within several days (Benson et al. 1907; Burton-Fanning 1901;

Table 3-1 Levels of Significant Exposure to Bromoform - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL: Less Serious (mg/kg/day)	LOAEL: Serious (mg/kg/day)	Reference Chemical Form
ACUTE EXPOSURE							
Death							
1	Human	1 d				445 F (single child died due to overdose)	Dwelle 1903
2	Rat (Sprague-Dawley)	1 day (GO)				1147 F (LD50)	Chu et al 1982a
3	Rat (Sprague-Dawley)	(G)				1388 M (LD50) 1147[b] F (LD50)	Chu et al. 1980
4	Rat (Fischer- 344)	1 d (GO)				933 (LD50)	NTP 1989a
5	Rat (Fischer- 344)	14 d 1x/d (GO)				600 (100% mortality)	NTP 1989a
6	Mouse (ICR)	1 d (GW)				1400[b] M (LD50) 1550 F (LD50)	Bowman et al 1978
7	Mouse (B6C3F1)	1 d (GO)				707[b] M (LD50) 1072 F (LD50)	NTP 1989a

Table 3-1 Levels of Significant Exposure to Bromoform - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
Systemic							
8	Rat (Fischer- 344)	14 d 1x/d (GO)	Endocr	600	800 (enlarged thyroid gland)		NTP 1989a
			Bd Wt	200 M	400 M (14% decreased body weight gain)		
9	Rat	1 d (G)	Hepatic	1440			Plaa and Hewitt 1982a
10	Rat (Fischer- 344)	7 d (GW)	Endocr	190 M	380 M (decreased serum testosterone)		Potter et al 1996
			Bd Wt	380 M			
11	Mouse (B6C3F1)	9 doses in 11 days (GO)	Hepatic		200 F (hepatocellular ballooning and proliferation)		Coffin et al 2000
12	Mouse (B6C3F1)	11 days (W)	Hepatic		300 F (hepatocellular ballooning)		Coffin et al 2000
13	Mouse (CD-1)	14 d 1x/d (GO)	Hepatic	72[c] M	145 M (centrilobular pallor)		Condie et al 1983
			Renal	72 M	145 M (mesangial nephrosis)		
			Bd Wt	289 M			

Table 3-1 Levels of Significant Exposure to Bromoform - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
14	Mouse (CD-1)	14 d 1x/d (GW)	Hepatic	50 M	125 M (increased absolute and relative liver weights)		Munson et al 1982
15	Mouse (B6C3F1)	14 d 1x/d (GO)	Gastro	200 M	400 M (stomach nodules)		NTP 1989a
Immuno/ Lymphoret							
16	Mouse (CD-1)	14 d 1x/d (GW)		125 M	250 M (impaired humoral immune function)		Munson et al 1982
Neurological							
17	Rat (Fischer- 344)	14 d 1x/d (GO)		400		600 (lethargy, labored and swallowing breathing, ataxia)	NTP 1989a
18	Rat (Fischer- 344)	1 d (GO)		500		1000 (shallow breathing)	NTP 1989a
19	Mouse (ICR)	14 days daily (GW)		9.7 M			Balster and Borzelleca 1982 Bromoform
20	Mouse (ICR)	1 d (GW)			431 M (ED50 for impaired motor performance)		Balster and Borzelleca 1982

Table 3-1 Levels of Significant Exposure to Bromoform - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
21	Mouse (ICR)	1 d (GW)				1000 (ataxia, sedation, anesthesia)	Bowman et al 1978
22	Mouse (B6C3F1)	14 d 1x/d (GO)		400		600 (lethargy and ataxia)	NTP 1989a
Developmental							
23	Rat (Sprague-Dawley)	9 d Gd 6-15 (GO)		100		200 (skeletal anom.)	Ruddick et al 1983
INTERMEDIATE EXPOSURE							
Systemic							
24	Rat (Wistar)	30 d (F)	Hepatic		56.4 F (hepatocellular vacuolization and swelling)		Aida et al 1992
			Bd Wt	187.2 M		617.9 M (24% decreased body weight)	
25	Rat (Sprague-Dawley)	28 d (W)	Hemato	14 M			Chu et al 1982a
			Hepatic	14 M			
			Renal	14 M			
			Bd Wt	14 M			

Table 3-1 Levels of Significant Exposure to Bromoform - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
26	Rat (Fischer- 344)	13 wk 5d/wk (GO)	Resp	200			NTP 1989a
			Cardio	200			
			Gastro	200			
			Hepatic	25 M[d]	50 M (hepatocellular vacuolization)		
			Renal	200			
			Endocr	200			
			Dermal	200			
			Bd Wt	200			
27	Mouse (B6C3F1)	3 wk 5 d/wk (GO)	Hepatic	200 F	500 F (hepatocyte hydropic degeneration; increased SGPT and sorbitol dehydrogenase)		Melnick et al 1998
			Bd Wt	500 F			

Table 3-1 Levels of Significant Exposure to Bromoform - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
28	Mouse (B6C3F1)	13 wk 5d/wk (GO)	Resp	400			NTP 1989a
			Cardio	400			
			Hepatic	100 M	200 M (minimal to moderate heptocellular vacuoles)		
			Renal	400			
			Endocr	400			
			Dermal	400			
			Bd Wt	400			
29	Mouse (Swiss)	102 d 1x/d (GO)	Resp	200			NTP 1989b
			Hepatic		200 (hepatocullular vacuoles)		
			Renal	200			
			Endocr	200			
Neurological							
30	Rat (Fischer- 344)	13 wk 5d/wk (GO)		50 M	100 M (lethargy)		NTP 1989a
31	Mouse (ICR)	90 d 1x/d (GW)		0.9 M	9.2 M (decreased exploratory activity)		Balster and Borzelleca 1982 Bromoform

Table 3-1 Levels of Significant Exposure to Bromoform - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
32	Mouse (ICR)	60 d 1x/d (GW)			100 M (decreased response rate in operant behavior test)		Balster and Borzelleca 1982 Bromoform
33	Mouse (ICR)	30d 1x/d (GW)		100 M			Balster and Borzelleca 1982
Reproductive							
34	Rat (Fischer- 344)	13 wk 5d/wk (GO)		200			NTP 1989a
35	Mouse (B6C3F1)	13 wk 5d/wk (GO)		400			NTP 1989a
36	Mouse (Swiss)	105 d 1x/d (GO)		200			NTP 1989b
CHRONIC EXPOSURE							
Death							
37	Rat (Fischer- 344)	103 wk 5d/wk (GO)				200 M (decreased survival)	NTP 1989a

Table 3-1 Levels of Significant Exposure to Bromoform - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
Systemic							
38	Rat (Fischer- 344)	103 wk 5d/wk (GO)	Cardio	200			NTP 1989a
			Gastro	100 M	200 M (forestomach ulcer)		
			Hemato	100 F	200 F (spleen pigmentation)		
			Hepatic		100[e] (hepatocellular vacuolization)		
			Renal	200			
			Endocr		100 M (pituitary gland hyperplasia)		
			Dermal	200			
			Bd Wt	100	200 (>10% decrease in body weight gain)		
39	Rat (Wistar)	2 year (F)	Hepatic	35 F	140 F (yellowing of liver; increased absolute and relative liver weight)		Tobe et al 1982
			Bd Wt	90 M		590 M (40% decrease in body weight gain)	

Table 3-1 Levels of Significant Exposure to Bromoform - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
40	Mouse (B6C3F1)	103 wk 5d/wk (GO)	Resp	100 M			NTP 1989a
			Cardio	100 M			
			Gastro	50 M	100 M (hyperplasia in glandular stomach)		
			Musc/skel	100 M			
			Hepatic		100 F (hepatocellular vacuolization)		
			Renal	100 M			
			Endocr	100 F	200 F (follicular cell hyperplasia in thyroid)		
			Dermal	100 M			
			Bd Wt	100 M			
Neurological							
41	Rat (Fischer- 344)	103 wk 5d/wk (GO)			100 (lethargy)		NTP 1989a
Reproductive							
42	Rat (Fischer- 344)	103 wk 5d/wk (GO)		100 M[b] 200 F	200 M (squamous metaplasia in prostate)		NTP 1989a

Table 3-1 Levels of Significant Exposure to Bromoform - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
43	Mouse (B6C3F1)	103 wk 5d/wk (GO)		100[b] M 200 F			NTP 1989a
Cancer							
44	Rat (Fischer- 344)	103 wk 5d/wk (GO)				200 F (CEL: adenomatous polyps and adenocarcinoma in large intestine)	NTP 1989a

a The number corresponds to entries in Figure 3-1.

b Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 3-1. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

c Used to derive an acute-duration oral MRL of 0.7 mg/kg/day; dose divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

d Used to derive an intermediate-duration oral MRL of 0.2 mg/kg/day; dose adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans, and 10 for human variability).

e Used to derive a chronic-duration oral MRL of 0.02 mg/kg/day; dose adjusted for intermittent exposure and divided by an uncertainty factor of 300 (3 for extrapolation from a minimal LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability) and a modifying factor of 10 to account for the identification of a lower LOAEL in a subchronic study (NTP 1989a).

d = day(s); F = female; (F) = food; (G) = gavage; Gastro = gastrointestinal; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; (W) = water; wk = week(s); x = time(s)

Figure 3-1. Levels of Significant Exposure to Bromoform - Oral

Acute (≤14 days)

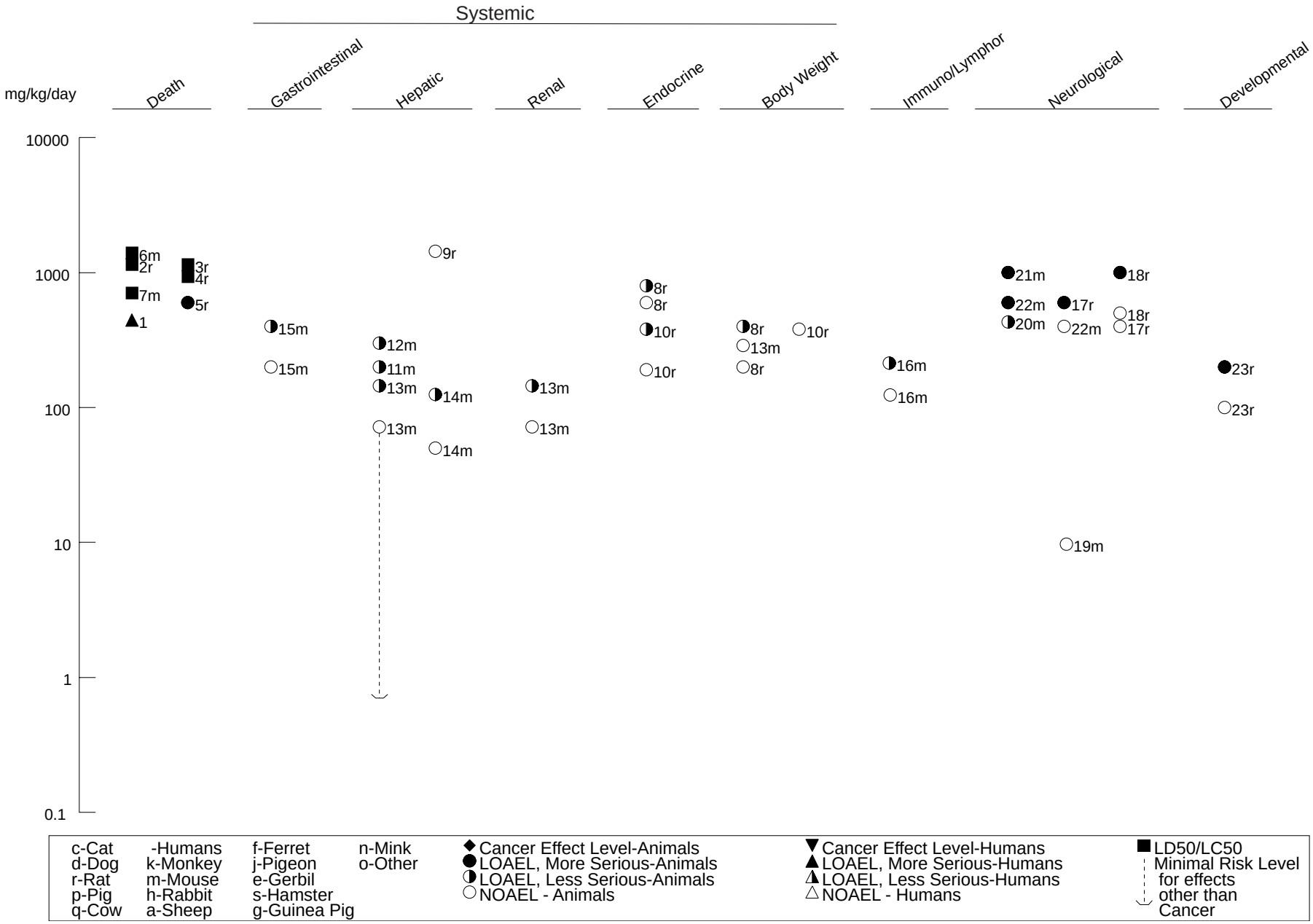


Figure 3-1. Levels of Significant Exposure to Bromoform - Oral (*Continued*)

Intermediate (15-364 days)

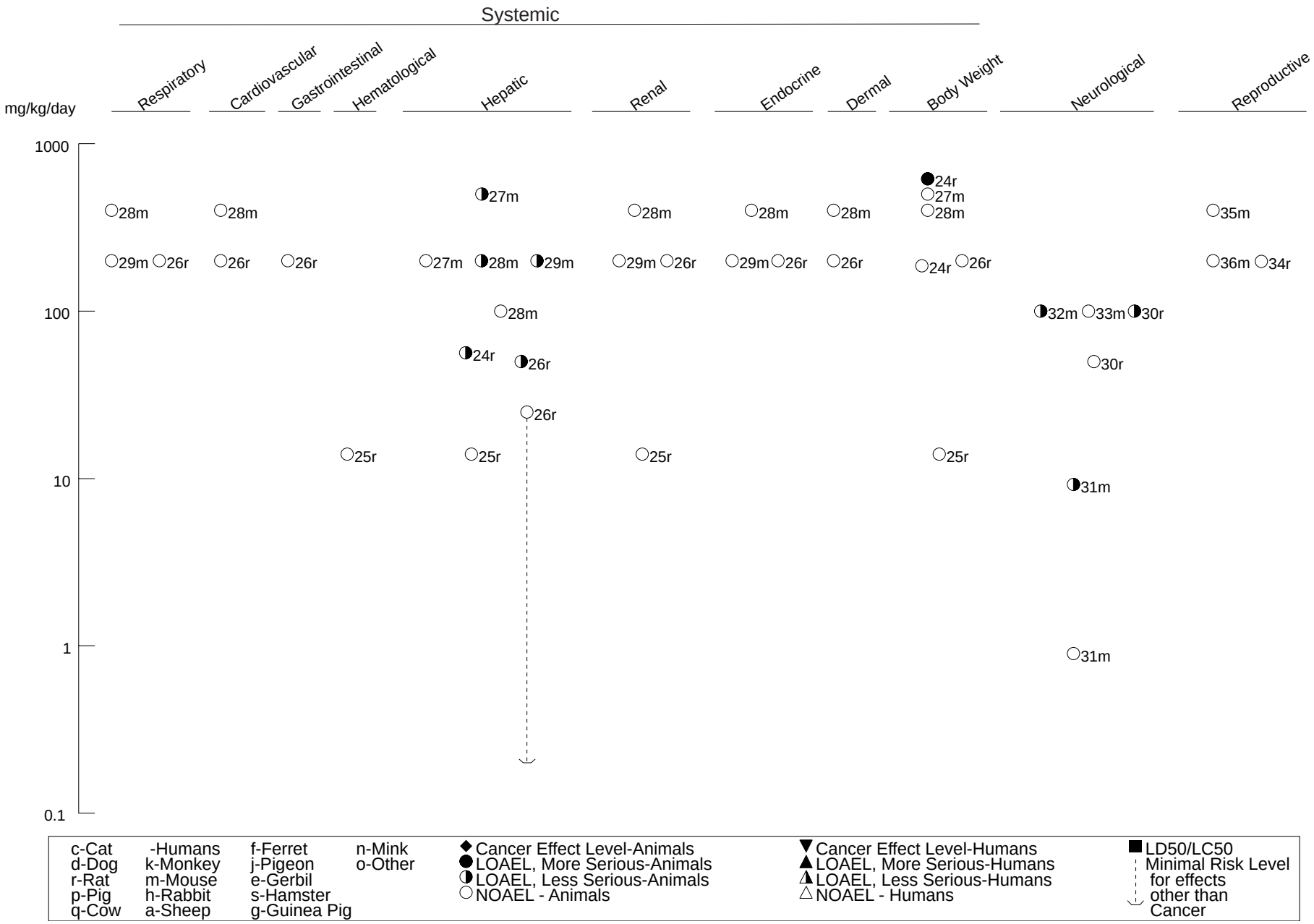


Figure 3-1 Levels of Significant Exposure to Bromoform - Oral

Chronic (≥365 days)

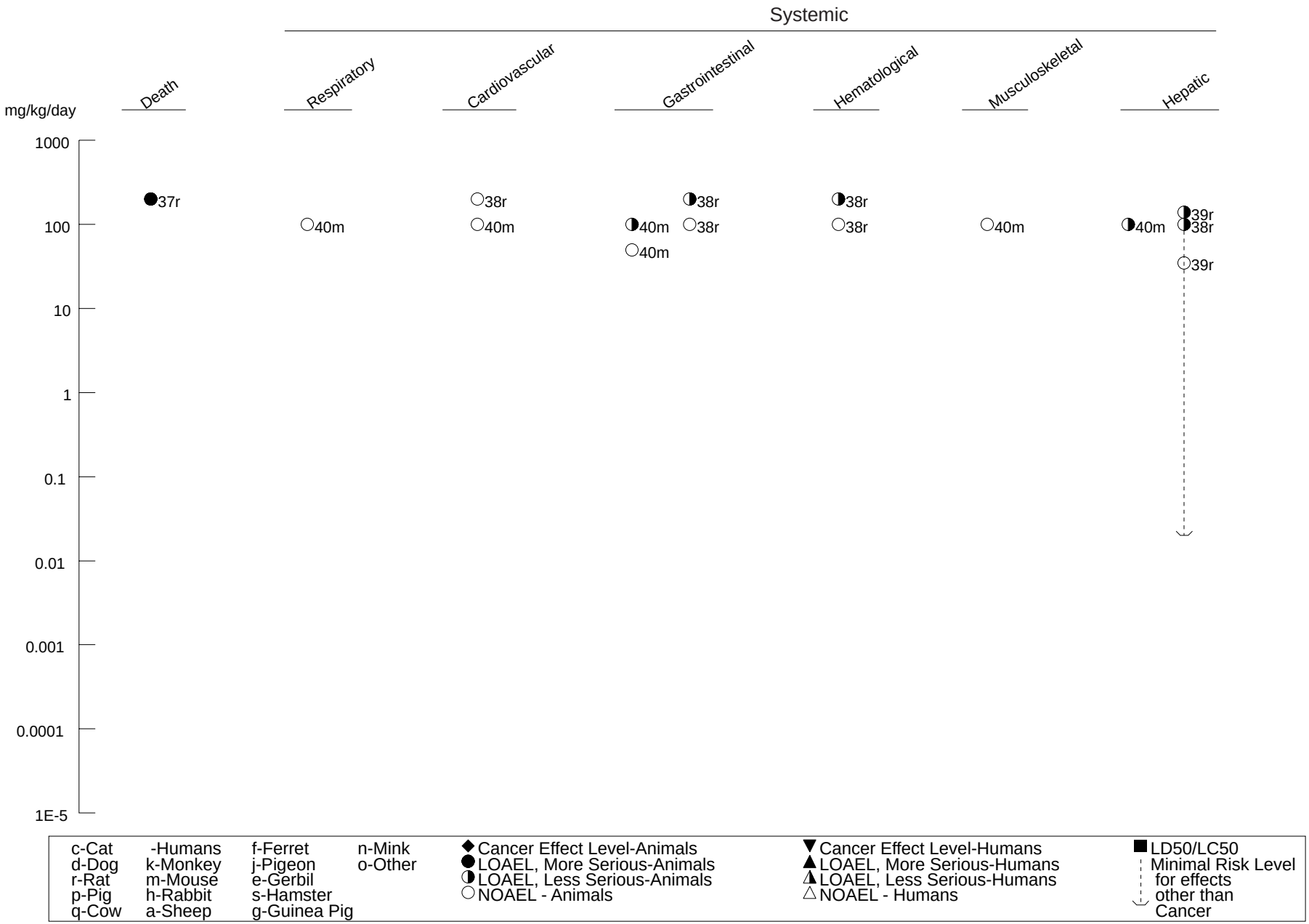


Figure 3-1 Levels of Significant Exposure to Bromoform - Oral

Chronic (≥365 days)

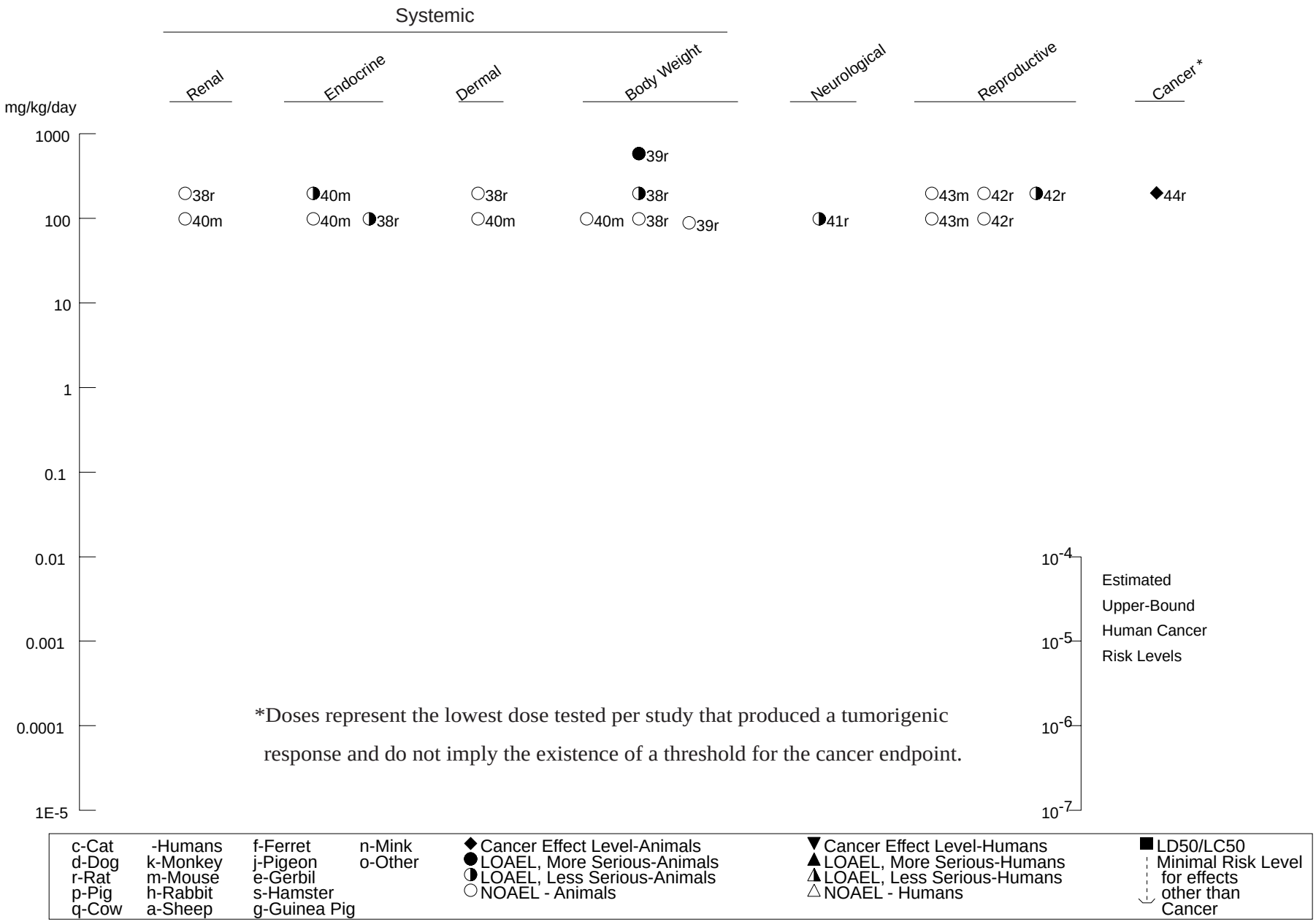


Table 3-2 Levels of Significant Exposure to Dibromochloromethane - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
ACUTE EXPOSURE							
Death							
1	Rat (Sprague-Dawley)	1 d (GO)				848 F (LD50)	Chu et al 1982a
2	Rat (Sprague-Dawley)	(G)				1186 M (LD50) 848[b] F (LD50)	Chu et al. 1980
3	Rat (Sprague-Dawley)	1 d (GO)				3700 M (100% mortality)	Hewitt et al 1983
4	Rat (Fischer- 344)	1 d (GO)				1250 M (4/5 died)	NTP 1985
5	Rat (Fischer- 344)	14 d 1x/d (GO)				500 (8 of 10 died)	NTP 1985
6	Mouse (ICR)	1 d (GW)				800[b] M (LD50) 1200 F (LD50)	Bowman et al 1978
7	Mouse (B6C3F1)	1 d (GO)				630 M (3/5 died)	NTP 1985
8	Mouse (B6C3F1)	14 d 1x/d (G)				500 (7 of 10 died)	NTP 1985

Table 3-2 Levels of Significant Exposure to Dibromochloromethane - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
9	Hamster (Golden Syrian)	1 d (G)				145 M (LD50)	Korz and Gatterman 1997
Systemic							
10	Rat (Sprague-Dawley)	1 d (GO)	Hepatic		2500 M (increased SGPT and OCT levels)		Hewitt et al 1983
			Renal	2500 M			
11	Rat (Fischer- 344)	14 d 1x/d (GO)	Hepatic	250	500 (mottled liver)		NTP 1985
			Renal	250	500 (darkened renal medullae)		
			Bd Wt	125 M		250 M (45% decrease body weight gain)	
12	Rat	1 d (G)	Hepatic	1220			Plaa and Hewitt 1982a
13	Rat (Fischer- 344)	7 d (GW)	Endocr	160 M	310 M (decreased serum testosterone)		Potter et al 1996
			Bd Wt	160 M	310 M (14% decrease in body weight)		

Table 3-2 Levels of Significant Exposure to Dibromochloromethane - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
14	Mouse (B6C3F1)	9 doses in 11 day period (GO)	Hepatic		100 F (hepatocellular ballooning and proliferation)		Coffin et al 2000
15	Mouse (B6C3F1)	11 days (W)	Hepatic		170 F (hepatocellular ballooning)		Coffin et al 2000
16	Mouse (CD-1)	14 d 1x/d (GO)	Hepatic		37[c] M (hepatocellular vacuolization)		Condie et al 1983
			Renal		37 M (mesangial hyperplasia)		
			Bd Wt	147 M			
17	Mouse (CD-1)	14 d 1x/d (GW)	Hepatic	125 F	250 F (increased relative and absolute liver weight, decreased serum glucose, and increased SGPT and SGOT)		Munson et al 1982
18	Mouse (B6C3F1)	14 d 1x/d (GO)	Renal	250	500 (reddened renal medullae)		NTP 1985
Immuno/ Lymphoret							
19	Mouse (CD-1)	14 d 1x/d (GW)		50 F	125 F (impaired humoral immunity)		Munson et al 1982

Table 3-2 Levels of Significant Exposure to Dibromochloromethane - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
Neurological							
20	Rat (Fischer- 344)	14 d 1x/d (GO)		250		500 (lethargy, ataxia)	NTP 1985
21	Rat (Fischer- 344)	1 d (GO)		160	310 (lethargy)		NTP 1985
22	Mouse (ICR)	14 days daily (GW)		10 M			Balster and Borzelleca 1982
23	Mouse (ICR)	1 d (GW)			454 M (ED50 for impaired motor performance)		Balster and Borzelleca 1982 Chlorodibromomethane
24	Mouse (ICR)	1 d (GW)				500 (sedation, anesthesia)	Bowman et al 1978
25	Mouse (B6C3F1)	14 d 1x/d (GO)		250		500 (lethargy, ataxia, and labored breathing)	NTP 1985
Developmental							
26	Rat (Sprague-Dawley)	9 d Gd 6-15 (GO)		200			Ruddick et al 1983
INTERMEDIATE EXPOSURE							
Death							
27	Rat (Fischer- 344)	13 wk 5d/wk (G)				250 (18/ 20 died)	NTP 1985

Table 3-2 Levels of Significant Exposure to Dibromochloromethane - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
Systemic							
28	Rat (Wistar)	30 d (F)	Hepatic	18.3 M	56.2 M (heptocellular vacuolation)		Aida et al 1992
			Bd Wt	173.3 M			
29	Rat (Sprague-Dawley)	28 d (W)	Hemato	12 M			Chu et al 1982a
			Hepatic	12 M			
			Renal	12 M			
			Bd Wt	12 M			

Table 3-2 Levels of Significant Exposure to Dibromochloromethane - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
30	Rat (Sprague-Dawley)	90 d (GO)	Resp	200			Daniel et al. 1990
			Cardio	200			
			Gastro	200			
			Hemato	200			
			Hepatic		50 (hepatocellular vacuolization)		
			Renal	50	100 (tubular degeneration)		
			Endocr	200			
			Dermal	200			
			Ocular	200			
			Bd Wt	50		200 (55% decrease in body weight gain)	

Table 3-2 Levels of Significant Exposure to Dibromochloromethane - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
31	Rat (Fischer- 344)	13 wk 5d/wk (GO)	Resp	250			NTP 1985
			Cardio	250			
			Gastro	250			
			Musc/skel	250			
			Hepatic	30 M	60 M (hepatocellular vacuolization)		
			Renal	125	250 (toxic nephropathy)		
			Endocr	250			
			Dermal	250			
			Bd Wt	125 M		250 M (47% decreased body weight gain)	
			Other		250 (salivary gland hyperplasia)		
32	Mouse (B6C3F1)	3 wk 5 d/wk (GO)	Hepatic	50 F	192 F (hepatocyte hydropic degeneration)		Melnick et al 1998
					100[b] F (increased relative liver weight)		
			Bd Wt	417 F			

Table 3-2 Levels of Significant Exposure to Dibromochloromethane - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
33	Mouse (B6C3F1)	13 wk 5d/wk (GO)	Resp	250			NTP 1985
			Gastro	250			
			Hepatic	125 M	250 M (hepatocellular vacuolization)		
			Renal	125 M	250 M (toxic nephropathy)		
			Endocr	250			
			Dermal	250			
			Bd Wt	250			
Immuno/ Lymphoret							
34	Rat (Sprague-Dawley)	90 d (GO)		100	200 (34-40% decr. in thymus wt.)		Daniel et al. 1990
Neurological							
35	Rat (Sprague-Dawley)	90 d (GO)		50 F	100 F (decreased absolute brain weight)		Daniel et al. 1990
36	Mouse (ICR)	60 d 1x/d (GW)		100 M	400 M (decreased response rate in operant behavior test)		Balster and Borzelleca 1982
37	Mouse (ICR)	30d 1x/d (GW)		100 M			Balster and Borzelleca 1982 Chlorodibromomethane

Table 3-2 Levels of Significant Exposure to Dibromochloromethane - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
Reproductive							
38	Rat (Sprague-Dawley)	90 d (GO)		100			Daniel et al. 1990
39	Rat (Fischer- 344)	13 wk 5d/wk (GO)		250			NTP 1985
40	Rat (Sprague-Dawley)	15 d (W)		47.8 F			NTP 1996
41	Rat (Sprague-Dawley)	28-34 d (W)		28.2 M 46 F			NTP 1996
42	Mouse (ICR)	2 generations (continuous) (W)		170 F		685 F (decreased fertility)	Borzelleca and Carchman 1982
43	Mouse (B6C3F1)	13 wk 5d/wk (GO)		250			NTP 1985
Developmental							
44	Mouse (ICR)	2 generations (continuous) (W)		685			Borzelleca and Carchman 1982

Table 3-2 Levels of Significant Exposure to Dibromochloromethane - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
CHRONIC EXPOSURE							
Death							
45	Mouse (B6C3F1)	105 wk 5d/wk (GO)				100 M (decreased survival)	NTP 1985
Systemic							
46	Rat (Fischer- 344)	2 yr 5d/wk (GO)	Resp	80			NTP 1985
			Cardio	80			
			Gastro	80			
			Musc/skel	80			
			Hepatic		40[d] (fatty change)		
			Endocr	80			
			Dermal	80			
			Bd Wt	80			
47	Rat (Wistar)	2 year (F)	Hepatic	20 M	85 M (yellowing of liver; hypertrophy)		Tobe et al 1982
			Bd Wt	20 M	85 M (10% decrease in body weight gain)	540 M (marked decrease in body weight gain)	

Table 3-2 Levels of Significant Exposure to Dibromochloromethane - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
48	Mouse (B6C3F1)	105 wk 5d/wk (GO)	Resp	100			NTP 1985
			Cardio	100			
			Gastro	100			
			Musc/skel	100			
			Hepatic		50 F (fatty metamorphosis)		
			Renal		100 M (nephrosis)		
			Endocr		50 F (thyroid follicular cell hyperplasia)		
			Dermal	100			
			Bd Wt	50	100 (14-17% decreased terminal body weight)		
Reproductive							
49	Rat (Fischer- 344)	2 yr 5d/wk (GO)		80			NTP 1985
50	Mouse (B6C3F1)	105 wk 5d/wk (GO)		100			NTP 1985

Table 3-2 Levels of Significant Exposure to Dibromochloromethane - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form
Cancer								
51	Mouse (B6C3F1)	105 wk 5d/wk (GO)				100	(CEL: hepatocellular adenoma or carcinoma)	NTP 1985

a The number corresponds to entries in Figure 3-2.

b Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 3-2. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

c Used to derive an acute-duration oral MRL of 0.1 mg/kg/day; dose divided by an uncertainty factor of 300 (3 for use of a minimal LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

d Used to derive a chronic-duration oral MRL of 0.09 mg/kg/day; dose adjusted for intermittent exposure and divided by an uncertainty factor of 300 (3 for use of a minimal LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

Bd Wt = body weight; CNS = central nervous system; d = day(s); F = female; (F) = food; (G) = gavage; Gd = gestation day; Gastro = gastrointestinal; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; (W) = water; wk = week(s); x = time(s)

Figure 3-2. Levels of Significant Exposure to Dibromochloromethane - Oral

Acute (≤14 days)

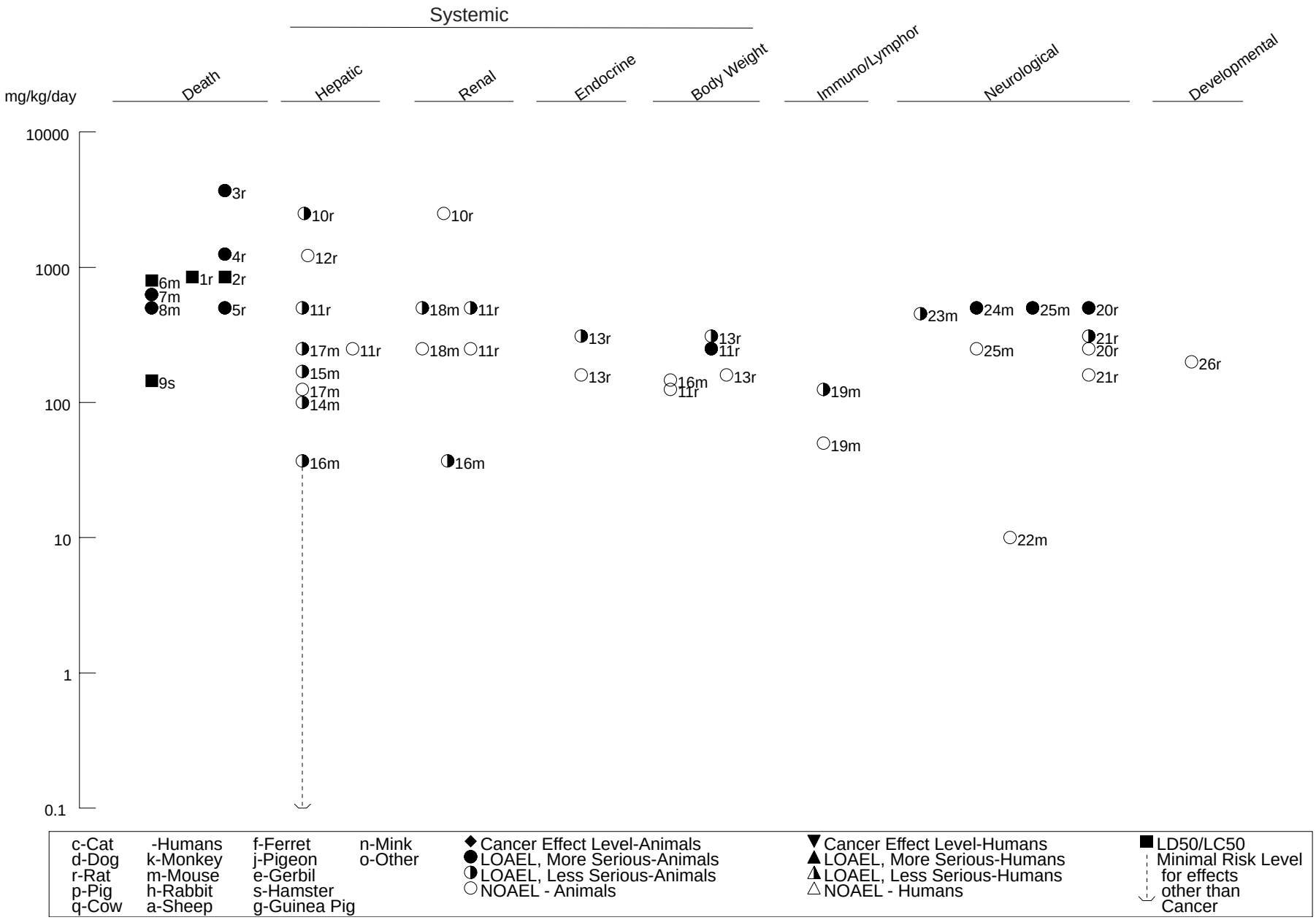


Figure 3-2. Levels of Significant Exposure to Dibromochloromethane - Oral (*Continued*)

Intermediate (15-364 days)

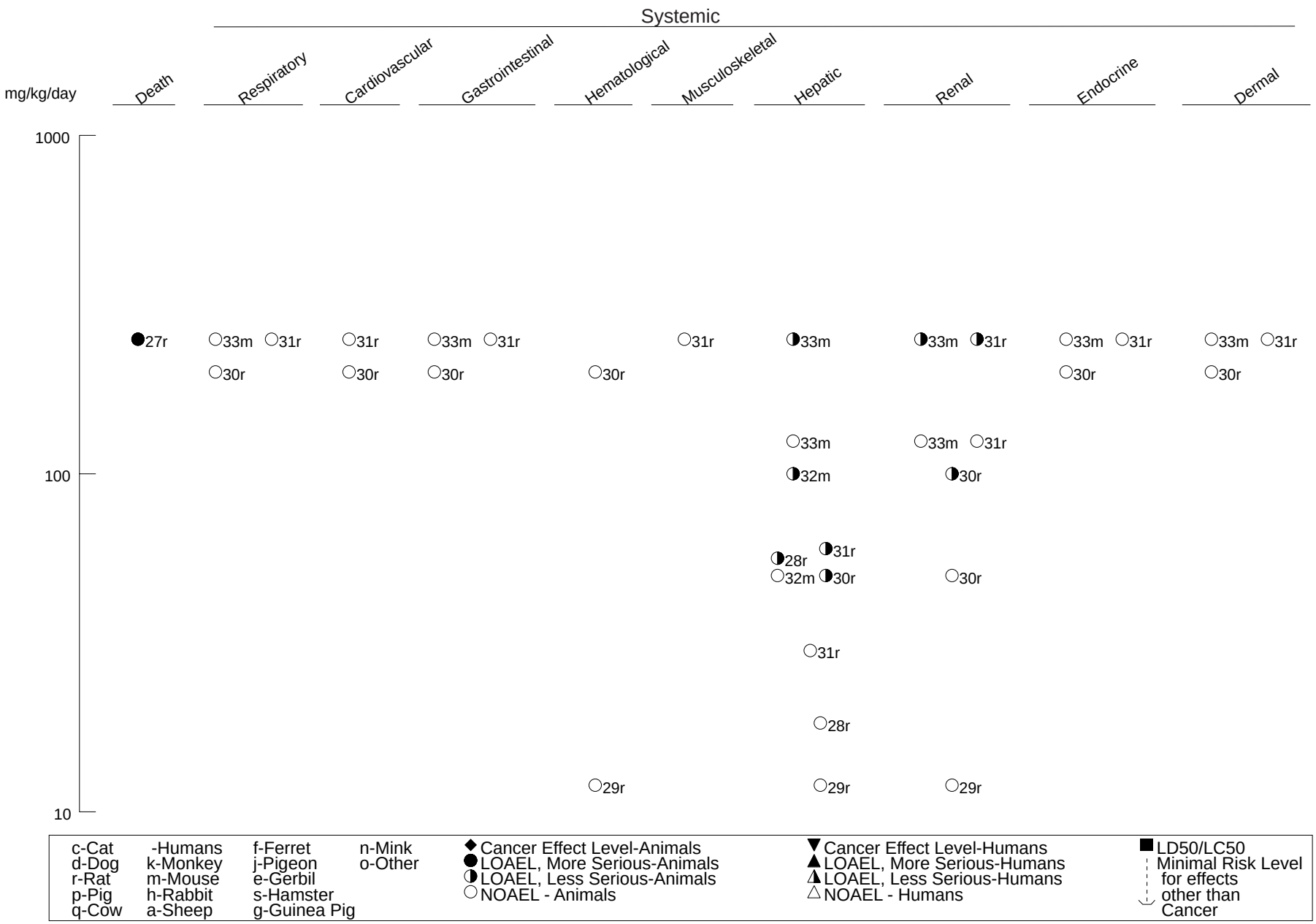


Figure 3-2. Levels of Significant Exposure to Dibromochloromethane - Oral (*Continued*)

Intermediate (15-364 days)

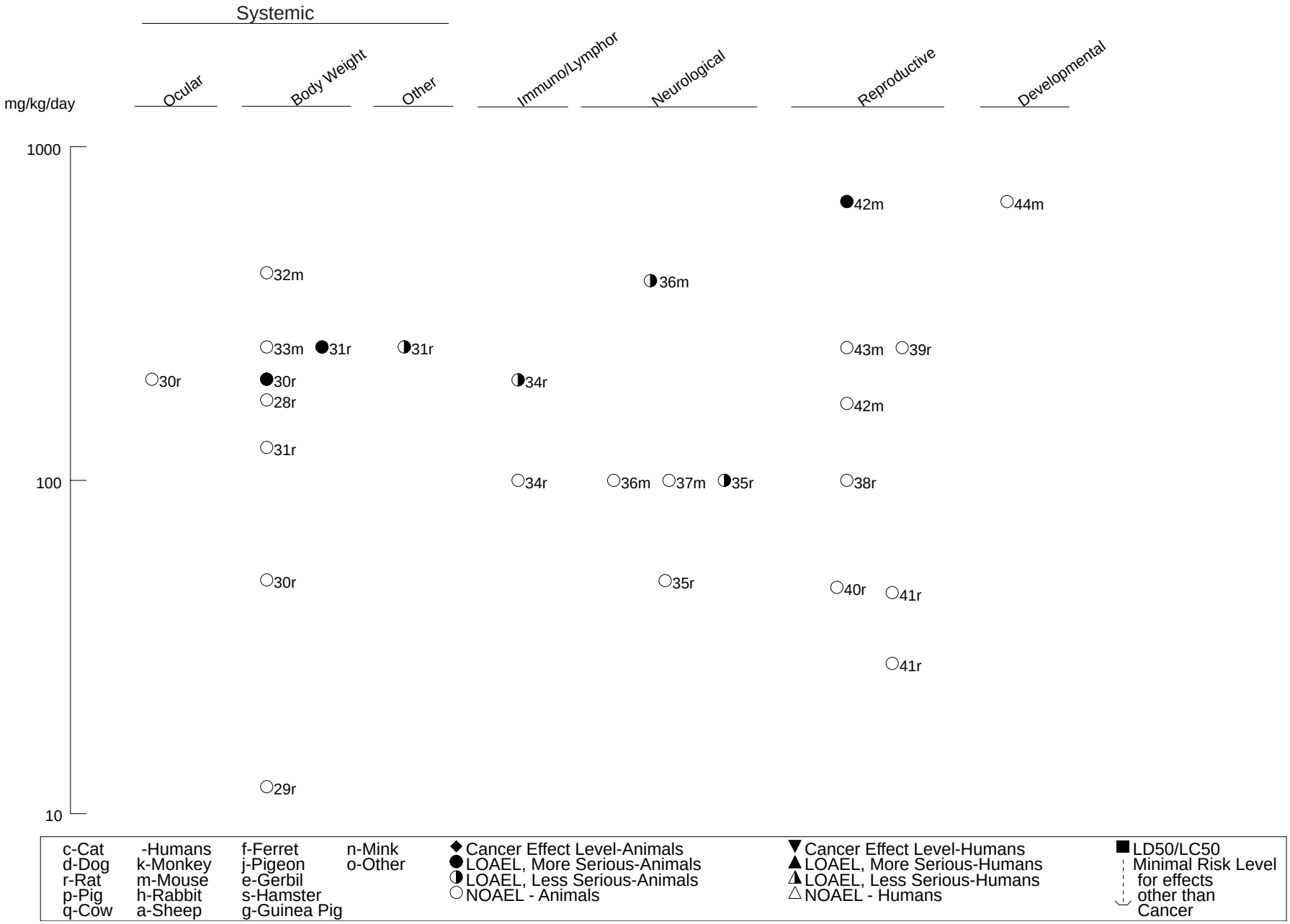
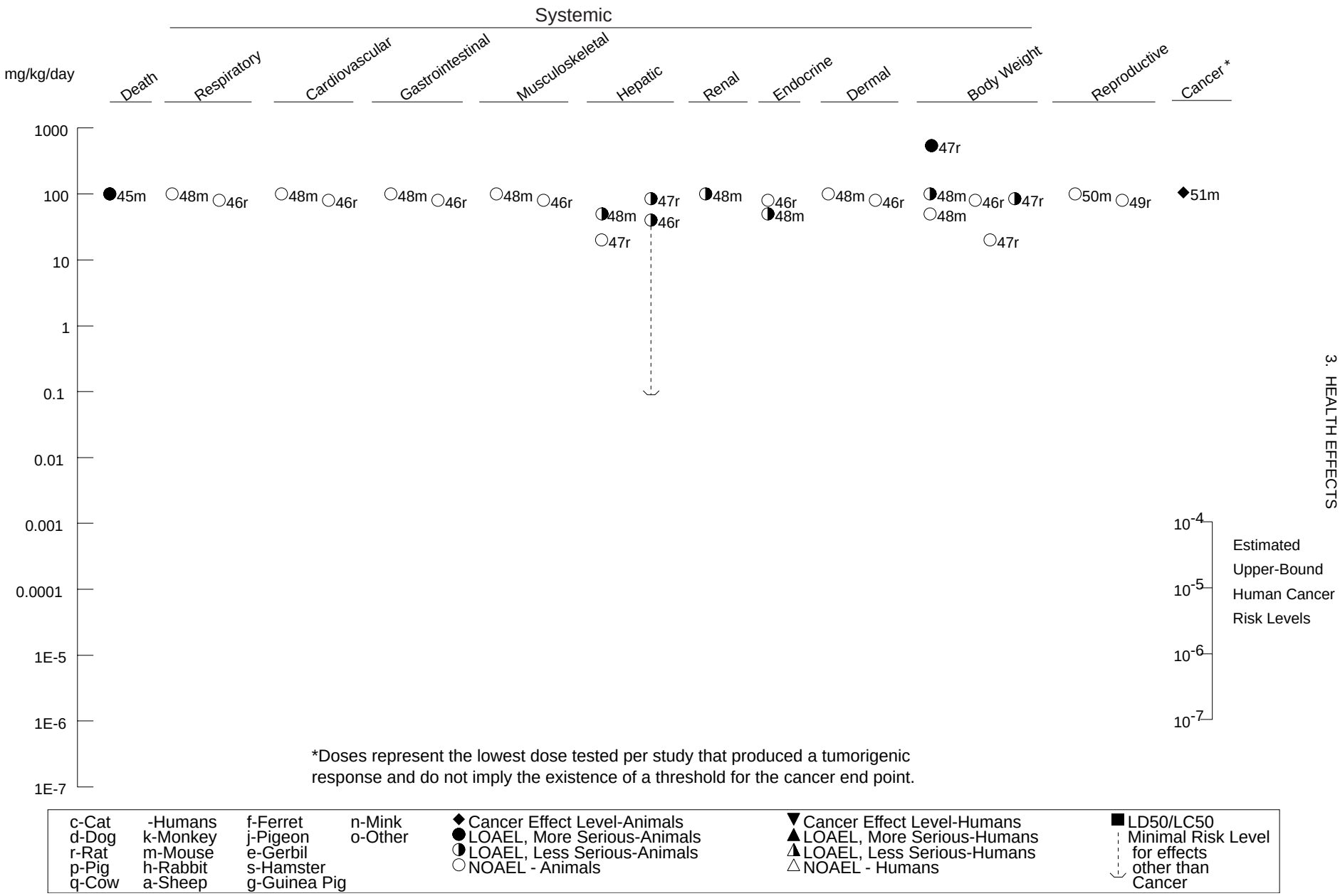


Figure 3-2. Levels of Significant Exposure to Dibromochloromethane - Oral (*Continued*)

Chronic (≥365 days)



*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer end point.

c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level for effects other than Cancer
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◭ LOAEL, Less Serious-Humans	
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	
q-Cow	a-Sheep	g-Guinea Pig				

Kobert 1906). The dose needed to cause death in children is not known with certainty, but both Dwelle (1903) and Roth (1904) estimated that a dose of about 5 g had been fatal. For a 10–20-kg child, this corresponds to an approximate dose of 250–300 mg/kg.

In animal studies, estimates of the acute oral LD_{50} for bromoform typically range between 707 and 1,550 mg/kg (Bowman et al. 1978; Chu et al. 1982a; NTP 1989a). There does not appear to be much difference in the doses that cause death across species or between sexes. The LD_{50} values for a 1-day exposure to bromoform ranged from 933 to 1,388 mg/kg in rats (Chu et al. 1980; NTP 1989a) and from 707 to 1,550 mg/kg in mice (Bowman et al. 1978; NTP 1989a). LD_{50} values ranged from 1,072 to 1,550 for females and from 707 to 1,388 for males exposed to bromoform for 1 day (Bowman et al. 1978; Chu et al. 1980, 1982a; NTP 1989a). Acute, repeated oral exposure to 600 mg/kg/day resulted in 100%mortality in rats (NTP 1989a). However, only 2/10 mice died from exposure to 800 mg/kg/day (NTP 1989a). No deaths were observed in rats and mice administered 200 or 400 mg/kg (5 days/week) via gavage for 90 days (NTP 1989a). However, significant reductions in survival were observed in rats administered 200 mg/kg via gavage (5 days/week) for 2 years (NTP 1989a).

In animals, the cause of death following acute oral exposure to bromoform has not been thoroughly investigated. Prominent clinical signs include central nervous system depression (Bowman et al. 1978). While central nervous system depression is probably an important factor in rapid lethality, some studies report death occuring several days after an acute exposure (Bowman et al. 1978; NTP 1989a). This suggests that other effects (e.g., hepatic and/or renal injury) may also be important.

Dibromochloromethane. No studies were located regarding death in humans after oral exposure to dibromochloromethane. The acute lethality of dibromochloromethane has been evaluated by several animal studies in rats and mice, with LD_{50} estimates ranging between 800 and 2,650 mg/kg (Bowman et al. 1978; Chu et al. 1980, 1982a; Hewitt et al. 1983; NTP 1985). Korz and Gatterman (1997) reported an LD_{50} of 145 mg/kg for a Golden Syrian hamster; however, the experimental details were not reported to validate the data. Deaths in animals have been reported from single oral doses as low 300–600 mg/kg (NTP 1985).

In intermediate and chronic oral exposure studies, significant increases in mortality have been observed in mice and rats at 250 mg/kg (5 days/week) for exposures of 14–90 days (Chu et al. 1982a; Condie et al. 1983; Daniel et al. 1990; NTP 1985) and in rats at 100 mg/kg (5 days/week) for exposures up to 2 years (NTP 1985).

Gender-related differences in mortality have been observed in rats and mice acutely exposured to dibromochloromethane. In rats, females appear to be more sensitive than males; increases in mortality were observed at 848 mg/kg in females compared to 1,186 mg/kg in males (Chu et al. 1982a). In contrast, increases in mortality were observed at lower doses in males (800 mg/kg) than females (1,200 mg/kg) (Bowman et al. 1978).

The cause of death following oral exposure of animals to dibromochloromethane has not been thoroughly investigated. Some of the chief clinical signs observed are those of central nervous system depression and other effects, such as hepatic and/or renal injury (Bowman et al. 1978; NTP 1985).

3.2.2.2 Systemic Effects

Respiratory Effects. Labored breathing has been observed in animals exposed to lethal doses of bromoform and dibromochloromethane (NTP 1985, 1989a); this is likely due to central nervous system depression rather than impaired lung function.

Bromoform. No studies were located regarding respiratory effects in humans after oral exposure to bromoform. NTP (1989a) examined the respiratory tract of rats and mice receiving gavage doses of bromoform for 90 days or 2 years. No histological alterations were observed in the intermediate-duration studies or in female rats and male and female mice in the chronic-duration study. An increased incidence of chronic inflammation of the lungs was observed in male rats exposed to 100 or 200 mg/kg (5 days/week). This inflammation was similar in appearance to that caused by a sialodacryoadenitis (SDA) virus infection, and antibodies to rat SDA virus were detected in study animals. Thus, the inflammation observed was probably secondary to the infection and was not a direct result of bromoform. However, the absence of symptoms in control animals suggested that bromoform-treated rats may have been more susceptible to infection by the virus or slower to recover (NTP 1989a).

Dibromochloromethane. No studies were located regarding respiratory effects in humans after oral exposure to dibromochloromethane. No histological alterations in the respiratory tract were observed in rats and mice administered gavage doses of up to 250 mg/kg (days/week) for 90 days or 80–100 mg/kg (5 days/week) (NTP 1985).

Cardiovascular Effects.

Bromoform. No studies were located regarding cardiovascular effects in humans after oral exposure to bromoform. Histological examination of rats and mice exposed to up to 100–200 mg/kg (5 days/week) bromoform by gavage for up to 2 years revealed no evidence of adverse effects upon the heart (NTP 1989a). While this indicates that cardiac tissue is not directly injured by bromoform, indirect effects on cardiovascular functions might occur as a consequence of the central nervous system depressant activity of these compounds.

Dibromochloromethane. No studies were located regarding cardiovascular effects in humans after oral exposure to dibromochloromethane. Decreased heart rates and increased blood pressure were observed in male rats following the administration of a single gavage dose (83–667 mg/kg) of dibromochloromethane (Müller et al. 1997). However, a dose-related trend was not exhibited for these. While this suggests that cardiac function may be impaired by dibromochloromethane exposure, it is unclear if effects are caused by the direct action on cardiac function or if they are indirectly caused as a result of central nervous system depression. No histological alterations to cardiac tissue were observed in intermediate- and chronic-duration studies of dibromochloromethane administered to rats and mice via gavage (NTP 1985).

Gastrointestinal Effects.

Bromoform. No studies were located regarding gastrointestinal effects in humans after oral exposure to bromoform. NTP (1989a) examined the esophagus, stomach, and intestines of rats and mice orally exposed to bromoform. Stomach nodules were observed in male and female mice following 14 days of exposure to 400 and 600 mg/kg/day, respectively (NTP 1989a). The biological significance of these nodules is not certain, but it is likely that they are a response to a direct irritant effect of bromoform on the gastric mucosa. In chronic-duration studies, histological alterations were observed in male rats and male mice. Forestomach ulcers were observed in male rats at 200 mg/kg (5 days/week) (NTP 1989a) and hyperplasia of the glandular stomach was observed in male mice at 100 mg/kg (5 days/week) (NTP 1989a). No stomach lesions were observed in female rats or mice exposed to bromoform at doses up to 200 mg/kg. The histological observations in NTP (1989a) suggest that males may be more sensitive to gastrointestinal effects from acute and chronic bromoform exposures than females. In acute studies, the onset of stomach nodules occurred at a lower dose level in males (400 mg/kg) than in females (600 mg/kg). In chronic studies, gastrointestinal effects were only observed in males. While these

observations clearly indicate that the stomach may be affected by bromoform, it is possible that the exposure regimen (bolus administration) leads to irritant effects in the stomach that might not occur if exposure were continuous at lower concentrations in food or drinking water.

Dibromochloromethane. No studies were located regarding gastrointestinal effects in humans after oral exposure to dibromochloromethane. No histological changes of the esophagus, stomach, or intestines were observed in mice or rats administered dibromochloromethane via gavage at doses up to 100 mg/kg (5 days/week) for up to 2 years (NTP 1985).

Hematological Effects. Several studies (Chu et al. 1982a, 1982b; Munson et al. 1982; Tobe et al. 1982) have investigated the hematological effects of oral exposure of rats and mice to bromoform and dibromochloromethane. With the exceptions of some minor fluctuations in lymphocyte count following exposure to bromoform (Chu et al. 1982a, 1982b), none of these studies detected any significant effects of bromoform or dibromochloromethane on hemoglobin, hematocrit, red blood cells, or white blood cells.

Musculoskeletal Effects. No studies regarding musculoskeletal effects in humans or animals after oral exposure to bromoform or dibromochloromethane.

Hepatic Effects. The liver is the primary target organ for bromoform and dibromochloromethane-induced toxicity. Oral exposure to these compounds results in an accumulation of fat in the liver, manifested as increased liver weight, appearence of hepatocyte vacuoles, alterations in serum cholesterol levels, and decreases in serum triglyceride levels. In addition to fatty liver changes, exposure to bromoform and dibromochloromethane results in focal hepatocellular necrosis. The necrosis is typically observed at higher doses than the fatty liver. The toxicity of both compounds is greater following gavage administration compared to exposure via the diet or drinking water. This is probably due to the large bolus dose overwhelming the liver's ability to detoxify reactive metabolites and the oil vehicle, which likely increases absorption.

Bromoform. Several animal studies have examined the hepatotoxicity of bromoform in rats and mice following gavage, drinking water, or dietary exposure. The most sensitive hepatic end point appears to be fatty degeneration as indicated by centrilobular pallor observed in mice at 145 mg/kg/day for 14 days (Condie et al. 1983) and hepatocellular vacuolization and/or swelling, which has been observed following acute gavage exposure to 200 mg/kg (9 days in an 11-day period) (Coffin et al. 2000), acute drinking water exposure to 300 mg/kg/day (Coffin et al. 2000), intermediate-duration gavage exposure to 50 mg/kg

(5 days/week) (NTP 1989a), dietary exposure to 56.4 mg/kg/day (Aida et al. 1992), and chronic gavage exposure to 100 mg/kg (5 days/week) (NTP 1989a). Increases in relative and/or absolute liver weights, although not always consistently found, occur at similar doses (Munson et al. 1982; Tobe et al. 1982). Alterations in clinical chemistry parameters associated with the fatty changes (alterations in serum triglyceride and cholesterol levels) are typically observed at higher doses. Decreases in serum triglyceride levels were observed at 207.5 and 590 mg/kg/day in rats exposed via the diet for 1 or 24 months, respectively (Aida et al. 1992; Tobe et al. 1982); the no effect levels for this end point are 56.4 and 90 mg/kg/day, respectively. An increase in serum total cholesterol was observed in rats exposed to ≥187.2 mg/kg/day in the diet for 1 month (Aida et al. 1992) and a decrease was found in rats exposed to 590 mg/kg/day in the diet for 24 months (Tobe et al. 1982). The data are inadequate to assess whether the difference in the direction of change was related to exposure duration or was an inconsistent finding.

Higher doses of bromoform result in hepatocellular necrosis. Necrosis was observed in rats exposed to 200 mg/kg (5 days/week) for 2 years (NTP 1989a); focal inflammation has also been observed in mice receiving gavage doses of 280 mg/kg for 14 days (Condie et al. 1983). Significant increases in SGPT and/or SGOT, which is indicative of hepatocellular damage, were observed in mice at 250–289 mg/kg/day for 14 days (Condie et al. 1983; Munson et al. 1982), mice at 500 mg/kg (5 days/week) for 3 weeks (Melnick et al. 1998), and rats at 720 mg/kg/day for 24 months (Tobe et al. 1982). A decrease in serum glucose levels observed in rats exposed to ≥61.9 mg/kg/day for 1 month (Aida et al. 1992) or ≥40 mg/kg/day for 24 months (Tobe et al. 1982) and an increase in hexabarbital sleep time observed in mice at 125 mg/kg/day for 14 days (Munson et al. 1982) are also indicative of liver damage.

There are limited data to assess species differences. The results of the NTP intermediate-duration study (NTP 1989a) suggest that rats may be more sensitive to the hepatotoxicity of bromoform than mice. The NOAEL and LOAEL values for hepatocellular vacuolization were 25 and 50 mg/kg (5 days/week) in rats and 100 and 200 mg/kg in mice. Liver damage was the critical end point used for the derivation of acute-, intermediate-, and chronic-duration oral MRLs for bromoform, as described in the footnotes for Table 3-1 and in Appendix A.

Dibromochloromethane. A variety of hepatic effects have been observed in rats and mice orally exposed to dibromochloromethane. Hepatocellular vacuolization and/or swelling were observed at the lowest adverse effect levels. The LOAEL values for this end point are ≥37 mg/kg in acutely exposed mice (Coffin et al. 2000; Condie et al. 1983), ≥50 mg/kg in rats and mice exposed for an intermediate duration (Aida et al. 1992; Daniel et al. 1990; NTP 1985), and ≥40 mg/kg in rats and mice following

chronic exposure (NTP 1985). Clinical chemistry alterations, which are associated with the fatty changes in the liver, include increases in serum cholesterol after a single gavage dose of 1,500 mg/kg (Chu et al. 1982a) or after a 1-month dietary exposure to 56.2 mg/kg/day (Aida et al. 1992), decreases in serum cholesterol at 540 mg/kg/day after a 24-month dietary exposure (Tobe et al. 1982), and decreases in serum triglycerides at 173.3 and 20 mg/kg/day following 1- or 24-month dietary exposures, respectively (Aida et al. 1992; Tobe et al. 1982). Additionally, increases in liver weight were observed in mice administered gavage doses of 250 mg/kg/day for 14 days (Munson et al. 1982) or 100 mg/kg (5 days/week) for 3 weeks (Melnick et al. 1998). The increased incidences of hepatocellular vacuoles observed at 37 mg/kg/day following acute exposure and 40 mg/kg following chronic exposure (NTP 1985) were used to derive acute- and chronic-duration oral MRLs for dibromochloromethane, as described in the footnotes to Table 3-2 and in Appendix A.

At higher doses, oral exposure to dibromochloromethane resulted in hepatocellular necrosis. Necrosis was found in rats and mice exposed to ≥100 mg/kg (Aida et al. 1992; Daniel et al. 1990; NTP 1985) for intermediate durations. Necrosis was not reported following acute-duration exposure; however, increases in SGOT and/or SGPT (indicative of hepatocellular damage) were found in rats and mice exposed to ≥145 mg/kg (Condie et al. 2000; Hewitt et al. 1983; Munson et al. 1982). Necrosis was not found following chronic exposure in rats and mice exposed to 80 or 100 mg/kg (5 days/week), respectively (NTP 1985). Other liver effects include bile duct hyperplasia in rats exposed to 250 mg/kg (5 days/week) for 13 weeks (NTP 1985), hepatocyte hydropic degeneration in mice exposed to 192 mg/kg (5 days/week) for 3 weeks (Melnick et al. 1998), and decreased serum glucose levels in rats exposed to 540 mg/kg/day for 24 months (Tobe et al. 1982).

Renal Effects.

Bromoform. There is some evidence that oral exposure to bromoform can induce kidney damage. Condie et al. (1983) noted minimal to slight nephrosis and mesangial hypertrophy in male mice exposed to repeated oral doses of 145–289 mg/kg/day of bromoform. In contrast, no significant histopathological alterations were detected by NTP (1989a) in rats or mice receiving gavage of 200 mg/kg (5 days/week) of bromoform for 2 years.

Dibromochloromethane. Histological studies performed by NTP (1985) indicate that oral exposure to dibromochloromethane can cause kidney injury in both rats and mice. The medullae appear to be reddened in both males and females after a single oral dose of 500 mg/kg, but this dose was so high that

7 of 10 animals died. Of greater toxicological concern were effects on the nephron that develop after intermediate or chronic exposure to doses of 50–250 mg/kg/day (NTP 1985). These effects were usually much more apparent in males than females, and were characterized by tubular degeneration and mineralization leading to nephrosis (NTP 1985). These histological findings of nephrotoxicity are supported by the kidney function studies of Condie et al. (1983), which found that ingestion of dibromochloromethane tended to impair uptake of para-amino hippuric acid in renal slices prepared from male mice exposed to 37–147 mg/kg/day for 2 weeks.

Endocrine Effects.

Bromoform. Several endocrine effects have been observed in animals exposed to bromoform; however, none of the effects were consistently found. The observed endocrine effects included enlarged thyroid gland in rats administered a lethal dose of 800 mg/kg/day for 14 days (NTP 1989a), decreased thyroid follicular size and colloid density 90 days after termination of exposure to 360 mg/kg/day (Chu et al. 1982b), thyroid follicular cell hyperplasia in mice administered 200 mg/kg (5 days/week) for 2 years (NTP 1989a), pituitary gland hyperplasia in rats administered 100 mg/kg (5 days/week), but not 200 mg/kg (NTP 1989a), and decreased serum testosterone levels in rats exposed to 380 mg/kg/day for 7 days (Potter et al. 1996).

Dibromochloromethane. Several studies have examined the potential of dibromochloromethane to induce histological alterations in endocrine glands following longer-term exposure. No alterations in endocrine glands were observed following intermediate-duration exposure of rats to 200 or 256 mg/kg/day (Chu et al. 1982b; Daniel et al. 1990) or rats or mice to 250 mg/kg for 5 days/week (NTP 1985). Administration of dibromochloromethane via gavage for 2 years resulted in thyroid follicular cell hyperplasia in mice exposed to 50 or 100 mg/kg (5 days/week). No effects were observed in rats administered 40 or 80 mg/kg (5 days/week) (NTP 1985). No effects on serum testosterone levels were observed in rats given gavage doses of 160 mg/kg/day for 7 days. However, a decrease in levels resulted from doses of 310 mg/kg/day (Potter et al. 1996).

Dermal Effects. No histological alterations were found in the skin of rats and mice exposed to bromoform or dibromochloromethane by gavage for up to 2 years (NTP 1985, 1989a).

Ocular Effects. No histological alterations were found in the eyes of rats and mice exposed to bromoform or dibromochloromethane by gavage for up to 2 years (NTP 1985, 1989a).

Body Weight Effects.

Bromoform. In animals, treatment with bromoform is associated with significant decrements in body weight, without concurrent reductions in food intake. The threshold for body weight effects appears to be duration-related, as generally observed in the oral exposure studies. In acute repeated-dose oral gavage studies (Condie et al. 1983; Munson et al. 1982, NTP 1989a), 4 and 14% decreases in body weight were observed in female and male rats, respectively, given 400 mg/kg. No significant, consistently-observed effects on body weight were seen in mice administered up to 800 mg/kg. Intermediate-duration studies of bromoform appear to present a similar dose-response relationship for body weight effects as the acute studies. Body weights of rats were unaffected by bromoform doses of ≤200 mg/kg administered by gavage, in feed, or in drinking water (Aida et al. 1992; Chu et al. 1982a; NTP 1989a). Body weights of mice treated with 400 mg/kg of bromoform by gavage were slightly (8%) reduced (NTP 1989a). Lower adverse effect levels were observed in chronic studies. Body weights were decreased by 4–10% in rats and female mice treated with 100 mg/kg of bromoform by gavage, whereas 16–25% decreases in body weight gain were noted at 200 mg/kg (NTP 1989a). A chronic feeding study in rats (Tobe et al. 1982) showed a dose-response similar to the gavage studies. Body weight reductions of 15% were observed at 90–150 mg/kg, while reductions of 30–40% were observed at doses above 365 mg/kg.

Dibromochloromethane. Treatment with dibromochlormethane is also associated with decreased body weight gain in animals. Rats appear to be more sensitive than mice to body weight effects. The dose-response relationship seemed to be sensitive to exposure duration, but was similar for varoius adminstration routes (gavage, feed, drinking water). The greater sensitiviy of rats to dibromochloro-methane compared with mice was appearent in both acute and intermediate duration exposures (Chu et al. 1980; Condie et al. 1983; Daniel et al. 1990; Munson et al. 1982; NTP 1985). Rats experienced 20% (400 mg/kg/day) and 47% (250 mg/kg/day) decreases in body weight gain from acute and intermediate gavage exposures, respectively. In contrast, no effect was seen in mice for these dose levels and durations. Duration-related changes to the dose-response were indicated in both species for body weight gain decreases of 10% or more. These effects on body weight gain occurred in the range of 250–500 mg/kg (acute), 200–250 mg/kg (intermediate), and 70–200 mg/kg (chronic) (Aida et al. 1992; Daniel et al. 1990; NTP 1985; Tobe et al. 1982). In chronic exposure studies (NTP 1985; Tobe et al. 1982), neither species nor administration route seemed to affect the dose response.

3.2.2.3 Immunological and Lymphoreticular Effects

There are limited data on the immune and lymphoreticular system toxicity of bromoform and dibromochloromethane. Munson et al. (1982) examined immune function following exposure to both compounds. Other studies (Daniel et al. 1990; NTP 1985, 1989a) monitored organ weights or examined tissues for histological damage.

Bromoform. Impaired humoral immune function, as indicated by the response to sheep red blood cells, was observed in rats and mice exposed to 125 or 250 mg/kg/day bromoform for 14 days (Munson et al. 1982), though no adverse effect on cell-mediated immunity was noted. Additionally, male rats exposed to bromoform for 2 years appeared to have decreased resistance to a common viral infection (NTP 1989a), suggesting functional impairment of the immune system. No histological alterations were observed in tissues of the immune or lymphoreticular systems in rats and mice exposed to 200 and 400 mg/kg (5 days/week), respectively, for 90 days (NTP 1985) or 100–200 or 200 mg/kg (5 days/week), respectively, for 2 years (NTP 1985).

Dibromochloromethane. Exposure of mice to doses of 125 or 250 mg/kg/day of dibromochloromethane for 14 days resulted in impaired humoral immunity (splenic IgM response to sheep red blood cells) (Munson et al. 1982). Impaired cellular immunity (popliteal lymph node response to sheep red blood cells) was also observed at 250 mg/kg/day. A significant decrease in thymus weight was observed in rats exposed to 300 mg/kg/day for 90 days (Daniel et al. 1990). No histological alterations were observed in immune or lymphoreticular tissues of rats and mice following intermediate or chronic duration to 250 or 80–100 mg/kg (days/week), respectively (NTP 1985).

3.2.2.4 Neurological Effects

Bromoform. Bromoform, like other volatile halogenated hydrocarbons, can lead to marked central nervous system depression. Because of this property, bromoform was used as a sedative in the early 1900s and was commonly administered to children for relief from whooping cough. Several poisonings and a few deaths resulted from accidental overdoses or separation of the emulsion (Benson 1907; Burton-Fanning 1901; Dwelle 1903; Stokes 1900; von Oettingen 1955). In mild cases of accidental overdose, clinical signs included rapid breathing, constricted pupils, and tremors; more severe cases were accompanied by a drunken-like stupor, cyanosis, shallow breathing, and erratic heart rate (Benson 1907; Kobert 1906). Actual doses associated with these neurological symptoms are not known with certainty.

Based on experiences with seven cases of accidental bromoform poisonings in children with whooping cough, Burton-Fanning (1901) advised initial treatment volumes of 0.03 mL for children under 1 year, 0.06 mL for those 1–4 years old, and 0.13 mL for children 4–8 years of age, or approximately 25–60 mg/kg. Dwelle (1903) reported the case of a 33-month-old girl who was prescribed bromoform for relief of whooping cough-induced coughing and vomiting. It is estimated that the girl ingested approximately 700 mg bromoform (60 mg/kg/day) and slept undisturbed that night, suggesting the occurrence of sedative effects.

As with humans, high doses of bromoform result in central nervous system depression in animals. Impaired performance on neurobehavioral tests is observed at lower doses,. The severity of the central nervous system depression is dose-related, with anesthesia and shallow breathing occurring at very high, often lethal, doses and ataxia, lethargy, and sedation at lower doses. In rats, lethargy, shallow breathing, and ataxia were observed at 600 and 1,000 mg/kg/day (NTP 1989a) following acute exposure. Lethargy was also observed at 100 mg/kg (5 days/week) during a 13-week or 2-year exposure (NTP 1989a). In mice, anesthesia, ataxia, and sedation were observed following a single dose of 1,000 mg/kg (Bowman et al. 1978) and lethargy and ataxia were observed following doses of 600 mg/kg/day for 14 days (NTP 1989a). No overt signs of neurotoxicity were observed at 100 or 400 mg/kg following gavage administration, 5 days/week, for 13 weeks or 2 years (NTP 1989a).

A series of experiments conducted by Balster and Borzelleca (1982) assessed neurobehavioral performance in mice following acute- or intermediate-duration exposures. The ED_{50} for impaired motor performance was 431 mg/kg following a single dose. A decrease in exploratory behavior was observed following 9.2 mg/kg/day for 90 days and a decrease in response rate in an operant behavior test following doses of 100 mg/kg/day for 60 days. No effect on swimming endurance was observed (9.7 mg/kg/day for 14 days) or on passive-avoidance learning (100 mg/kg/day for 30 days).

Dibromochloromethane. No human data on the neurotoxicity of dibromochloromethane were located. In animals, central nervous system depression occurs at relatively high doses, as evidenced by lethargy, ataxia, and sedation. An acute exposure to 500 mg/kg/day resulted in lethargy, ataxia, sedation, and shallow breathing in rats and mice (Bowman et al. 1978; NTP 1985). Lethargy was also observed in rats receiving a single gavage dose of 310 mg/kg (NTP 1985). No overt signs of neurotoxicity were observed following intermediate-duration exposure to 250 mg/kg (5 days/week) or chronic exposure to 80–100 mg/kg (5 days/week) (NTP 1985). Daniel et al. (1990) found a significant decrease in brain weights in rats exposed to 100 or 200 mg/kg/day for 90 days. However, no alterations in brain weight or

histopathology were observed at doses as high as 250 mg/kg (5 days/week) for 13 weeks (NTP 1985) or 80 mg/kg (5 days/week) for 2 years (NTP 1985).

Some alterations in neurobehavioral performance were observed in mice (Balster and Borzelleca 1982). An ED_{50} of 454 mg/kg was calculated for impaired motor performance following a single dose exposure and a decrease in response rate in operant behavior test during a 60-day exposure to 400 mg/kg/day. No alterations were observed in swimming endurance (10 mg/kg/day for 14 days), exploratory behavior (10 mg/kg/day for 90 days), or passive avoidance learning tests (100 mg/kg/day for 30 days).

3.2.2.5 Reproductive Effects

The reproductive toxicity of bromoform and dibromochloromethane has been assessed in a small number of human and animal studies. A number of studies have examined the potential association between adverse reproductive outcomes (spontaneous abortions, stillbirths, and preterm delivery) and consumption of municipal drinking water containing triholomethanes (bromoform, dibromochloromethane, dichloromethane, and chloroform) (Bove et al. 1992, 1995, 2002; Dodds et al. 1999; Kramer et al. 1992; Mills et al. 1998; Nieuwenhuijsen et al. 2000; Savitz et al. 1995; Waller et al. 1999). These studies involved mixed exposures to trihalomethane compounds and most did not provide bromoform or dibromochloromethane exposure data. Two pregnancy outcome studies (Kramer et al. 1992; Waller et al. 1999) have examined risks associated with levels of bromoform or dibromochloromethane in drinking water. A third study (Windham et al. 2003) examined the association of altered menstrual cycle function in women exposed to trihalomethanes in drinking water. Collectively, the studies provide insufficient evidence for establishing a causal relationship between exposure to trihalomethane compounds and adverse reproductive outcome.

The Kramer et al. (1992) study is a population case-control study of pregnant white, non-Hispanic women living in communities in Iowa where all drinking water was supplied from a single source. No significant associations were found between bromoform or dibromochloromethane levels in the drinking water and the risk of prematurity. The odds ratios were 1.1 (95% confidence limit of 0.8–1.4) for communities with bromoform levels of 1 μg/L and higher and 1.1 (95% confidence limit of 0.7–1.4) for communities with dibromochloromethane levels of 1–3 μg/L. No cases were found in communities with dibromochloromethane levels of 4 μg/L and higher. Similar results were found in the Waller et al. (1999) study. In this study, pregnancy health outcomes were examined in pregnant women living in communities with groundwater, surface water, or mixed sources of drinking water. The risk of spontaneous abortion was

not significantly associated with bromoform or dibromochloromethane water levels; the percentage of pregnancies ending with spontaneous abortions were 9.2, 9.8, and 10.3% in communities with bromoform drinking water levels of 0, 1–15, and ≥16 μg/L, respectively, and 9.7, 9.6, and 10.4% for dibromochloromethane levels of 0, 1–30, and ≥31 μg/L, respectively.

Windham et al. (2003) examined the possible association between trihalomethanes in drinking water and menstrual cycle function. Menstrual parameter values in premenopausal women were determined based on hormone levels in urine collected during an average of 5.6 menstrual cycles (n=403). Estimates of bromoform and dibromochloromethane levels for each cycle were based on residential data (individual trihalomethane concentrations measured within a relatively narrow time period around the menstrual cycle start date) and utility measurements (quarterly measurements made by water utilities during a 90-day period beginning 60 days before the cycle start date). Significant associations were found between exposure to the top quartile of bromoform (≥12 μg/L) and decreasing menstrual cycle and follicular phase length. The age, race, body mass index, income, pregnancy history, and caffeine and alcohol consumption-adjusted differences were -0.79 days (95% confidence interval [CI] of -1.4 to -0.14) for cycle length and -0.78 days (95% CI -1.4 to -0.14) for follicular phase length. For dibromochloromethane, the differences for the 2–3 quartile (level not reported) and highest quartile (≥20 μg/L) were -0.69 days (95% CI of -1.4 to -0.02) and -1.21 days (95% CI of -2.0 to -0.38), respectively, for menstrual cycle length and -0.62 days (95% CI of -1.3 to 0.05) and -1.1 days (95% CI of -1.9 to -0.25), respectively, for follicular phase length. No associations between bromoform or dibromochloromethane concentration and luteal phase length, menses length, or cycle variability were found. Interpretation of the study results is limited due to coexposure to other trihalomethanes.

Bromoform. Data on the reproductive toxicity of bromoform in humans are limited to the trihalomethane studies described above. Animal studies have examined the potential of bromoform to induce histological alterations (NTP 1989a) and impair reproductive function (NTP 1989b). No histological alterations were observed in rats and mice exposed to 200 or 400 mg/kg, respectively, 5 days/week for 13 weeks (NTP 1989a). Dose related incidences of squamous metaplasia of the prostate gland were observed in male rats exposed to 200 mg/kg (5 days/week) for 2 years (NTP 1989a). This lesion may represent a chemical effect associated with concurrent inflammatory lesions in this gland, which occurred at similar rates in all groups of male rats tested, including vehicle controls. Chronic exposure of mice to 200 mg/kg (5 days/week) resulted in no detectable noncancerous histological effects in male reproductive tissues (testes, prostate, and seminal vesicles) (NTP 1989a). No nonneoplastic histological alterations were observed in female rats or mice receiving gavage doses of 200 mg/kg (5 days/week) for 2 years (NTP

1989a). No adverse effects on reproductive performance or fertility were observed in male and female mice receiving gavage doses of 200 mg/kg/day (NTP 1989b).

Dibromochloromethane. Human data on the reproductive toxicity of dibromochloromethane are limited to the trihalomethane studies described above. No histological alterations (testes, prostate, seminal vesicles, ovaries, uterus, and mammary gland examined) were observed in rats or mice receiving gavage doses of up to 250 mg/kg 5 days/week for 13 weeks (Daniel et al. 1990; NTP 1985) or 80 or 100 mg/kg, respectively, 5 days/week for 2 years (NTP 1985). In contrast to these negative findings, female mice exposed to dibromochloromethane in drinking water at a high dose (685 mg/kg/day) experienced a marked reduction in fertility, with significant decreases in litter size, gestational survival, postnatal survival, and postnatal body weight (Borzelleca and Carchman 1982). These effects may have been due to marked maternal toxicity, as evidenced by decreased weight gain, enlarged and discolored livers, and decreased survival. Exposure to lower doses (17 or 170 mg/kg/day) resulted in occasional decreases in one or more of the reproductive parameters monitored, but the effects were not large and were not clearly dose-related. These data are not sufficient to draw firm conclusions about the effects of dibromochloromethane on reproduction, but it appears that reproductive tissues and functions are not markedly impaired at doses that do not cause frank maternal toxicity. This is supported by a reproductive toxicity study conducted by NTP (1996). No alterations in reproductive or fertility indices were observed in female rats exposed to 40.3 mg/kg/day dibromochloromethane in drinking water for 35 days (13 days prior to mating, during mating, and gestation).

3.2.2.6 Developmental Effects

There are limited data on the developmental toxicity of bromoform or dibromochloromethane in humans and animals. Several human studies have examined the potential association between exposure to trihalomethanes in drinking water and birth outcomes (Dodds et al. 1999; Kramer et al. 1992; Savitz et al. 1995). However, only one study (Kramer et al. 1992) examined the exposure to individual trihalomethanes. In this population-based case control of pregnant white, non-Hispanic women living in communities in Iowa where all drinking water was supplied from a single source, no significant association between exposure to ≥1 μg/L of bromoform or ≥4 μg/L of dibromochloromethane in tap water and increased risk of low birth weight babies and intrauterine growth retardation were found. Interpretation of this study is limited by the co-exposure to other trihalomethane compounds.

Bromoform. Human data on the developmental toxicity of bromoform are limited to the trihalomethane studies discussed above. One animal study (Ruddick et al. 1983) was identified. No significant alterations in the number of resorption sites, fetuses per litter, fetal body weights, fetal malformations, or visceral anomalies were observed in the offspring of rats administered up to 200 mg/kg/day bromoform in corn oil on gestational days 6–15. Increases in several skeletal anomalies were found in the offspring of rats exposed to bromoform, including the presence of a 14th rib, wavy ribs, interparietal deviations, and sternebra aberrations. The study authors did not conduct a statistical analysis of the data. However, an independent analysis of the data using Fisher Exact test revealed a significant increase in the incidence (per fetus and number of affected litters) in sternebra aberrations.

Dibromochloromethane. Human data on the developmental toxicity of dibromochloromethane are limited to the trihalomethane studies discussed above. Two animal studies examined the potential of dibromochloromethane to induce developmental effects. In a study by Ruddick et al. (1983) of rats receiving gavage doses of up to 200 mg/kg/day on gestational days 6–15, no alterations in the number of resorption sites, fetuses per litter, fetal body weights, fetal gross malformations, or skeletal or visceral anomalies were found. Borzelleca and Carchman (1982) exposed mice to 685 mg/kg/day of dibromochloromethane in drinking water for several generations and detected no significant effect on the incidence of gross, skeletal, or soft-tissue anomalies.

3.2.2.7 Cancer

No studies were located regarding carcinogenic effects in humans following oral exposure to bromoform or dibromochloromethane. There are a number of epidemiological studies that indicate that there may be an association between chronic ingestion of chlorinated drinking water (which typically contains trihalomethanes including bromoform and dibromochloromethane) and increased risk of rectal, bladder, or colon cancer in humans (Cantor et al. 1987; Crump 1983; Kanarek and Young 1982; Marienfeld et al. 1986), but these studies cannot provide information on whether any effects observed are due to bromoform, dibromochloromethane, or to one or more of the hundreds of other byproducts that are also present in chlorinated water.

Bromoform. A significant increase in the incidence of adenomatous polyps or adenocarcinomas was observed in female rats receiving gavage dose of 200 mg/kg, 5 days/week for 2 years (NTP 1989a); a nonstatistically significant increase was observed in male rats. The incidences of this relatively rare tumor (combined incidences of adenomatous polyps and adenocarcinoma) were 0/50, 0/50, and 3/50 for

males administered 0, 100, or 200 mg/kg and 0/50, 1/50, and 8/50 for females. No significant alterations in neoplastic lesions were observed in mice administered up to 100 (males) or 200 (females) mg/kg, 5 days/week for 2 years (NTP 1989a). The International Agency for Research on Cancer (IARC) concluded that there were inadequate human data and limited animal data and assigned bromoform to weight of evidence category 3, not classifiable as to carcinogenicity in humans (IARC 1991a, 1999a) and EPA classified bromoform as a probable human carcinogen, group B2 (IRIS 2004a). Based on the increased occurrence of neoplastic lesions in the large intestines of female rats, EPA derived an oral slope factor of 7.9×10^{-3} $(mg/kg/day)^{-1}$ (IRIS 2004a).

Dibromochloromethane. In mice administered gavage doses of 100 mg/kg, 5 days/week for 2 years, increases in the incidences of hepatocellular adenomas and carcinomas were observed (NTP 1985). The incidence was significantly elevated for hepatocellular adenomas in females (2/50, 4/49, and 11/50 for 0, 50, and 100 mg/kg), hepatocellular carcinoma in males (10/50 and 19/50 for 0 and 100 mg/kg) and combined incidences for hepatocellular adenoma or carcinoma (23/50 and 27/50 for males at 0 and 100 mg/kg and 6/50, 10/49, and 19/50 for females at 0, 50, and 100 mg/kg). No significant alterations in the incidence of neoplastic lesion were observed in male or female rats administered up to 80 mg/kg 5 days/week for 2 years (NTP 1985). The IARC considered the available data on dibromochloromethane carcinogenicity to be not classifiable as to carcinogenicity in humans (group 3) (IARC 1991b, 1999b). EPA classified dibromochloromethane as a possible human carcinogen, group C (IRIS 2004b). Based on the increased combined incidence of hepatocellular adenoma or carcinoma in female mice, EPA derived an oral slope factor of 8.4×10^{-2} $(mg/kg/day)^{-1}$ (IRIS 2004b).

3.2.3 Dermal Exposure

No studies were located regarding the following health effects in humans or animals after dermal exposure to bromoform or dibromochloromethane:

3.2.3.1 Death

3.2.3.2 Systemic Effects

3.2.3.3 Immunological and Lymphoreticular Effects

3.2.3.4 Neurological Effects

3.2.3.5 Reproductive Effects

3.2.3.6 Developmental Effects

3.2.3.7 Cancer

3.3 GENOTOXICITY

Bromoform. The *in vivo* and *in vitro* genotoxicities of bromoform are examined in numerous studies, the results of which are summarized in Tables 3-3 and 3-4, respectively.

The potential of bromoform to induce gene mutations have reported have shown mixed results in *in vitro* assays. Increases (LeCurieux et al. 1995; Simmon and Tardiff 1978; Simmon et al. 1977) and no effect (Kubo et al. 2002; NTP 1989a; Rapson et al. 1980; Varma et al. 1988) on the occurrence of reverse mutations have been found in *Salmonella tymphimurium*; an increase in forward mutations was also found in *S. tymphimurium* (Roldan-Arjona and Pueyo 1993). The clastogenic activity of bromoform has been tested in one study (Galloway et al. 1985; NTP 1989a) that found no significant alterations with S9 metabolic activation and negative and weakly positive results without metabolic alteration. A significant alteration in mitotic aneuploidy was observed in *Aspergillus nidulans* (Benigni et al. 1993). Other tests of genotoxicity included an increase in sister chromatid exchange in human lymphocytes (Morimoto and Koizumi 1983) and rat erythroblastic leukemia K_3D cells (Fujie et al. 1993), but not in Chinese hamster ovary cells (Galloway et al. 1985; NTP 1989a) or oyster toadfish leukocytes (Maddock and Kelly 1980); SOS induction in *Escherichia coli* (Lecurieux et al. 1995); trifluorotymidine resistance in mouse lymphoma cells (NTP 1989a). DNA strand breaks were observed in human lymphocytes (Landi et al. 1999) and lymphoblastic leukemia cells, but not in primary rat hepatocytes (Geter et al. 2003a).

The potential for bromoform to induce chromosome aberrations, micronuclei, sister chromatid exchange, and DNA damage was investigated in several *in vivo* studies, often with conflicting results. An increase in chromosome aberrations, in particular chromatid and chromosome breaks, was observed in rats receiving five daily gavage doses of 253 mg/kg/day or a single intraperitoneal dose of 25.3 or 253 mg/kg (Fujie et al. 1990), but not in mice administered up to 800 mg/kg via intraperitoneal injection (NTP 1989a). A significant increase in sister chromatid exchange was observed in the bone marrow cells of mice receiving 25 mg/kg/day gavage doses for 4 days (Morimoto and Koizumi 1983) and mice receiving

Table 3-3. Genotoxicity of Bromoform *In Vivo*

End point	Species (test system)	Exposure route	Results	Reference
Nonmammalian systems:				
Sex-linked recessive lethal	*Drosophilia melanogaster*	Feeding	+	Woodruff et al. 1985; NTP 1989a
		Injection	–	
Reciprocal translocation	*D. melanogaster*	Feeding	–	Woodruff et al. 1985; NTP 1989a
Micronuclei	*Pleurodeleswaltl larvae*		+	LeCurieux et al. 1995
Mammalian systems:				
Sister chromatid exchange	Mouse (bone marrow cell)	IP	+	NTP 1989a
	Mouse (bone marrow cell)	oral	+	Morimoto and Koizumi 1983
Chromosomal aberrations	Mouse (bone marrow cell)	IP	–	NTP 1989a
	Rat (bone marrow cell)	IP	+	Fujie et al. 1990
	Rat (bone marrow cell)	Oral	+	Fujie et al. 1990
Micronuclei	Mouse (bone marrow cell)	IP	+	NTP 1989a
	Mouse (bone marrow cell)	Oral	–	Stocker et al. 1996
	Mouse (bone marrow cell)	IP	–	Hayashi et al. 1988
Repairable DNA damage	Rat (liver cells)	Oral	–	Stocker et al. 1996
DNA strand breaks	Rat (kidney cells)	Oral	–	Potter et al. 1996
	Rat (liver, kidney, duodenum)	Oral	–	Geter et al. 2003a

+ = positive result; – = negative result; IP = intraperitoneal

Table 3-4. Genotoxicity of Bromoform *In Vitro*

End point	Species (test system)	Results[a] With activation	Results[a] Without activation	Reference
Prokaryotic organisms:				
Reverse gene mutation	*Salmonella tymphimurium*			Simmon and Tardiff 1978; Simmon et al. 1977
	TA100	No data	+	
	TA1535	No data	+	
	S. typhimurium TA100	–	+	LeCurieux et al. 1995
	S. typhimurium TA100	–	–	Kubo et al. 2002
	TA98	–	–	
	S. typhimurium			Varma et al. 1988
	TA98	–	–	
	TA100	–	(+)	
	TA1535	–	–	
	TA1537	–	–	
	S. typhimurium TA100	No data	–	Rapson et al. 1980
	S. typhimurium (preincubation assay)			
	TA97	(+)	–	NTP 1989a
	TA98	–, –, (+)	–, –, –	
	TA100	–, –, –	(+), –, –	
	TA1535	–, –, –	–, –, –	
	TA1537	–, –, –	–, –, –	
Forward gene mutation	*S. typhimurium*	No data	(+)	Roldan-Arjona and Pueyo 1993
SOS induction	*Escherichia coli*	+	+	LeCurieux et al. 1995
Eukaryotic organisms:				
Fungi:				
Mitotic aneuploidy	*Aspergillus nidulans*	No data	+	Benigni et al. 1993
Fish:				
Sister-chromatid exchange	Oyster toadfish leukocytes	No data	–	Maddock and Kelly 1980
Mammalian cells:				
Sister-chromatid exchange	Human lymphocytes	No data	+	Morimoto and Koizumi 1983
	Chinese hamster ovary cells	–, –	–, (+)	Galloway et al. 1985; NTP 1989a

Table 3-4. Genotoxicity of Bromoform *In Vitro*

End point	Species (test system)	Results[a] With activation	Results[a] Without activation	Reference
	Rat erythroblastic leukemia K_3D cells	+	+	Fujie et al. 1993
Chromosomal aberrations	Chinese hamster ovary cells	–, –	–, (+)	Galloway et al. 1985; NTP 1989a
Trifluorothymidine resistance	Mouse lymphoma cells	+	+	NTP 1989a
DNA strand breaks	Human lymphocytes	No data	+	Landi et al. 1999
	Human lymphoblastic leukemia cells	+	+	Geter et al. 2003a
	Primary rat hepatocyte	–	–	Geter et al. 2003a

[a]Results from two or more different contract laboratories are separated by commas
+ = positive result; – = negative result; (+) = marginally positive result

intraperitoneal doses of 800 mg/kg (NTP 1989a). Increases in micronuclei induction were observed in mice exposed to 800 mg/kg via intraperitoneal injection (NTP 1989a) and newt larvae following a 6-day exposure to 2.5 mg/L (LeCurieux et al. 1995), but not in mice receiving a single intraperitoneal dose of 175–1,400 mg/kg (Hayashi et al. 1988) or 250–1,000 mg/kg via gavage (Stocker et al. 1997). No alterations in unscheduled DNA synthesis were observed in mice administered 324 or 1,080 mg/kg via gavage (Stocker et al. 1997). No alterations in DNA strand breaks were observed in the kidneys of rats administered 380 mg/kg for 1 day via gavage (Potter et al. 1996) or in the liver, kidney, or duodenum of rats administered 152 mg/kg either as a single oral bolus or on drinking water for 2 weeks (Geter et al. 2003a). An increase in sex-linked recessive mutations was observed in *Drosophila melanogaster* following feeding, but not injection exposure (NTP 1989a); no effect on reciprocal translocations was found.

Dibromochloromethane. The genotoxicity of dibromochloromethane has been assessed in a number of *in vivo* and *in vitro* assays. The results of these studies are presented in Tables 3-5 and 3-6, respectively.

Mixed results were found in *in vitro* bacterial assays for reverse gene mutations, with some studies finding significant alterations (Landi et al. 1999; Simmon and Tardiff 1978; Simmon et al. 1977; Varma et al. 1988) and others not finding an effect (Kubo et al. 2002; LeCurieux et al. 1995; NTP 1985; Zeiger et al. 1987). A significant increase in forward gene mutations was found in mouse lymphoma cells (McGregor et al. 1991). An increase in gene conversion, but not gene reversion, was observed in *Saccharomyces cervisiae* (Nestman and Lee 1985). An increase in mitotic aneuploidy was observed in *A. nidulans* and sister chromatid exchange was observed in human lymphocytes (Morimoto and Koizumi 1983; Sobti 1984), rat liver cells (Sobti 1984), and rat erythroblastic leukemia K_3D cells (Fuije et al. 1993). DNA strand breaks were observed in human lymphoblastic leukemia cells, but not in primary rat hepatocytes (Geter et al. 2003a). Additionally, increases in chromosomal aberrations were observed in mouse lymphoma cells (Sofuni et al. 1996) and Chinese hamster lung cells (Matsuoka et al. 1996).

In vivo, oral exposure to 25–200 mg/kg/day for 4 days resulted in dose-related increases in the frequency of sister chromatid exchanges in mouse bone marrow cells (Morimoto and Koizumi 1983). An increase in the occurrence of chromosomal aberrations was also observed in bone marrow cells of rats receiving a single intraperitoneal injection of 20.8 mg/kg (Fujie et al. 1990); a weak positive response was observed following gavage administration of 10.5–1,041.5 mg/kg/day for 5 days (Fujie et al. 1990). No significant alterations in micronuclei induction (Hayashi et al. 1988), repairable DNA damage (Stocker et al. 1996),

Table 3-5. Genotoxicity of Dibromochloromethane *In Vivo*

End point	Species (test system)	Exposure route	Results	Reference
Nonmammalian systems:				
Micronuclei	*Pleurodeleswaltl larvae*		–	LeCurieux et al. 1995
Mammalian systems:				
Sister chromatid exchange	Mouse (bone marrow cell)	Oral	+	Morimoto and Koizumi 1983
Chromosomal aberrations	Rat (bone marrow cell)	IP	+	Fujie et al. 1990
	Rat (bone marrow cell)	Oral	(+)	Fujie et al. 1990
Micronuclei	Mouse (bone marrow cell)	IP	–	Hayashi et al. 1988
Repairable DNA damage	Rat (liver cells)	Oral	–	Stocker et al. 1996
DNA strand breaks	Rat (kidney cells)	Oral	–	Potter et al. 1996
	Rat (liver, kidney, duodenum)	Oral	–	Geter et al. 2003a

+ = positive result; – = negative result; (+) = weak positive; IP = intraperitoneal

Table 3-6. Genotoxicity of Dibromochloromethane *In Vitro*

End point	Species (test system)	Results[a] With activation	Results[a] Without activation	Reference
Prokaryotic organisms:				
Reverse gene mutation	*Salmonella typhimurium* (desiccator sytstem)			Simmon and Tardiff 1978; Simmon et al. 1977
	TA100	No data	+	
	S. typhimurium			LeCurieux et al. 1995
	TA100	–	–	
	S. typhimurium			Kubo et al. 2002
	TA100	–	–	
	TA98	–	–	
	S. typhimurium (plate incorporation assay)			Varma et al. 1988
	TA98	+	–	
	TA100	+	–	
	TA1535	+	+	
	TA1537	+	+	
	S. typhimurium (preincubation assay)			NTP 1985; Zeiger et al. 1987
	TA98	–	–	
	TA100	–	–	
	TA1535	–	–	
	TA1537	–	–	
	S. typhimurium			Landi et al. 1999
	TPT100	+	+	
	RSJ100	–	–	
Eukaryotic organisms:				
Fungi:				
Mitotic aneuploidy	*Aspergillus nidulans*	No data	+	Benigni et al. 1993
Gene conversion	*Saccharomyces cervisiae*	–	+	Nestman and Lee 1985
Gene reversion	*S. cerevisiae*	–	–	Nestman and Lee 1985
Mammalian cells:				
Forward gene mutation	Mouse L5178Y lymphoma cells	No data	+	McGregor et al. 1991
Sister-chromatid exchange	Human lymphocytes	No data	+	Morimoto and Koizumi 1983
	Human lymphocytes	No data	+	Sobti 1984
	Rat liver cells	No data	(+)	Sobti 1984
	Rat erythroblastic leukemia K_3D cells	+	+	Fujie et al. 1993

Table 3-6. Genotoxicity of Dibromochloromethane *In Vitro*

End point	Species (test system)	Results[a] With activation	Results[a] Without activation	Reference
Chromosomal aberrations	Mouse L5178Y lymphoma cells	+,+	(+),–	Sofuni et al. 1996
	Chinese hamster lung cells	No data	+	Matsuoka et al. 1996
DNA single strand breaks	Human lymphoblastic leukemia cells	+	+	Geter et al. 2003a
	Priamry rat hepatocyte	–	–	Geter et al. 2003a

[a]Results from two or more different contract laboratories are separated by commas

+ = positive result; – = negative result; (+) = marginally positive result

or DNA strand breaks (Potter et al. 1996) were observed in mouse bone marrow cells (intraperitoneal injection of 62.5–1,000 mg/kg), rat liver cells (gavage dose of 135 or 450 mg/kg), or rat kidney cells (gavage dose of 310 mg/kg), respectively. Likewise, no DNA strand breaks were observed in the liver, kidney, or duodenum of rats administered 125 mg/kg either as a single oral bolus or on drinking water for 2 weeks (Geter et al. 2003a).

3.4 TOXICOKINETICS

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

There are limited data on inhalation absorption of bromoform or dibromochloromethane. Based on the physical-chemical properties of these compounds, and by analogy with other related halomethanes such as chloroform (Agency for Toxic Substances and Disease Registry 1997), it is expected that bromoform and dibromochloromethane would be well-absorbed across the lung. The occurrence of systemic and neurological effects following inhalation exposure of animals to bromoform (see Section 3.2.1) supports this view. Aggazzotti et al. (1998) examined the uptake of trihalomethanes in five swimmers exposed to trihalomethanes via chlorinated pool water. Although bromoform was detected in the pool water, it was only detected in one of four indoor air samples. Dibromochloromethane was detected in the pool water (0.8 μg/L) and in air samples before swimming (5.2 $\mu g/m^3$) and after swimming (11.4 $\mu g/m^3$). After sitting near the pool for 1 hour, the mean alveolar air level was 0.8 $\mu g/m^3$. After swimming, alveolar air levels were 1.4 $\mu g/m^3$; however, oral and percutaneous exposure also influenced this level. Estimated dibromochloromethane uptake during the 1 hour of sitting near the pool ranged from 1.5 to 2.0 μg/hour; after 1 hour of swimming, the estimated uptake rate ranged from 14 to 22 μg/hour.

Several studies have examined the impact of showering with water contaminated with trihalomethanes on the absorption of individual trihalomethane compounds. Showering involves inhalation exposure to volatilized trihalomethanes, percutaneous exposure, and possibly oral exposure. Several studies have found elevated blood bromoform and/or dibromochloromethane levels following showering (Backer et al. 2000; Lynberg et al. 2001; Miles et al. 2002). A comparative study by Backer et al. (2000) found the highest blood levels of dibromochloromethane after a 10-minute shower, compared to bathing for 10 minutes or drinking 1 L of water in 10 minutes. This difference may be due to differences in total exposure levels, metabolism, or excretion rather than differences in absorption efficiencies.

Several studies have estimated the blood:air partition coefficients for bromoform and dibromochloromethane in humans and rats, these values are summarized in Table 3-7.

3.4.1.2 Oral Exposure

There are limited data on the absorption of bromoform and dibromochloromethane following oral exposure. Most of the available data (Backer et al. 2000; da Silva et al. 1999; Mink et al. 1986) involved exposure to a mixture of trihalomethanes. Elevated blood concentrations of dibromochloromethane were also observed in humans ingesting drinking water containing trihalomethanes (Backer et al. 2000). da Silva et al. (1999) examined bromoform and dibromochloromethane absorption in rats following a single gavage dose. Both compounds were rapidly absorbed with peak plasma levels occurring <1 hour postexposure. Oral absorption constants of 0.412 and 0.55 per hour were reported for bromoforom and dibromochloromethane, respectively. A nonlinear relationship between dose (0.25 and 0.50 mmol/kg; 63 and 126 mg/kg bromoform and 52 and 104 mg/kg dibromochloromethane) and areas under the blood concentration versus time curves (AUCs) were found, suggesting metabolism saturation. The AUCs (20–360 minutes postexposure) for 63 and 126 mg/kg bromoform were 48.6 and 190.4 μM/hour; for 52 and 104 mg/kg dibromochlormethane, the AUCs (20–360 minutes) were 31.2 and 85.6 μM/hour. When bromoform and dibromochloromethane were administered along with chloroform and dichlorobromomethane (0.25 mmol/kg of each trihalomethane), the AUC was significantly higher than when administered singly.

Mink et al. (1986) found that 60–90% of the bromoform and dibromochloromethane administered in a mixture of trihalomethanes in corn oil to rats (100 mg trihalomethane/kg) or mice (150 mg trihalomethane/kg) were recovered in expired air, urine, or in internal organs. This indicates that gastrointestinal absorption was at least 60–90% complete. This is consistent with the ready gastrointestinal absorption observed for other halomethanes such as chloroform (Agency for Toxic Substances and Disease Registry 1997). As noted by Withey et al. (1983), the rate of halocarbon uptake from the gastrointestinal tract may be slower when compounds are given in oil than when they are given in water.

3.4.1.3 Dermal Exposure

No studies were located regarding dermal absorption of bromoform. A single study was found to quantitatively demonstrate dermal absorption of dibromochloromethane in human volunteers exposed for

Table 3-7. Partition Coefficients for Bromoform and Dibromochloromethane

	Chemical	Partition coefficient	Species	Reference
Blood:Air				
	Bromoform	102.3	Human	Batterman et al. 2002
		161	Rat	Beliveau and Krishnan 2000
		187	Rat	Beliveau et al. 2000b
		198.1	Rat	da Silva et al. 1999
	Dibromochloromethane	52.7	Human	Gargas et al. 1989
		49.2	Human	Batterman et al. 2002
		116	Rat	Gargas et al. 1989
		97.5	Rat	Beliveau et al. 2000b
Fat:Air				
	Bromoform	4,129	Rat	da Silva et al. 1999
	Dibromochloromethane	1,919	Rat	Gargas et al. 1989
Liver:Air				
	Bromoform	210.3	Rat	da Silva et al. 1999
	Dibromochloromethane	126	Rat	Gargas et al. 1989
Muscle:Air				
	Bromoform	115.1	Rat	da Silva et al. 1999
	Dibromochloromethane	55.6	Rat	Gargas et al. 1989

60 minutes to tap water having a temperature similar to that of bathing water (Prah et al. 2002). The sealed exposure apparatus was designed to permit the exposure of a single hand and lower arm by immersion while preventing an inhalation exposure. The mean tap water concentration of dibromochloromethane was 1.4 ng/mL. Dibromochloromethane levels in the blood increased constantly from the start to 5 minutes after the end of the exposure. At 15 minutes after cessation of exposure, blood levels appeared to fall slightly slower than the rate of appearance. These data have limited use since rates of dermal absorption or appearance in the blood were not reported.

As discussed under Inhalation Exposure, showering, which involves both inhalation and dermal exposure with water contaminated with trihalomethanes, can result in significantly elevated blood bromoform and dibromochloromethane levels. Xu et al. (2002) reported dermal absorption coefficients of 0.21 and 0.20 cm/hour for aqueous solutions of bromoform and dibromochloromethane, respectively. These values were determined *in vitro* using excess breast skin tissues at two temperature settings (20 and 25 °C).

3.4.2 Distribution

Several studies have estimated the tissue:air partition coefficients for bromoform and dibromochloromethane in humans and rats; these values are summarized in Table 3-7. *In vitro* data, suggest that hemoglobin is the primary ligand for bromoform, and presumably dibromochloromethane, in the blood (Béliveau and Krishnan 2000b).

3.4.2.1 Inhalation Exposure

No studies were located regarding the distribution of bromoform or dibromochloromethane in humans or animals following inhalation exposure. However, adverse effects involving several organs (liver, kidney, central nervous system) indicate distribution to these sites.

3.4.2.2 Oral Exposure

The distribution of bromoform and dibromochloromethane in tissues following oral exposure has not been thoroughly investigated. Analysis of bromoform levels in the organs of a child who died after an accidental overdose revealed concentrations of 10–40 mg/kg tissue in intestine, liver, kidney, and brain,

with somewhat higher levels in lung (90 mg/kg) and stomach (130 mg/kg) (Roth 1904, as cited in von Oettingen 1955). This suggests that bromoform is distributed fairly evenly from the stomach to other tissues.

In animals, Mink et al. (1986) found that only about 1–2% of a single oral dose of ^{14}C-labeled dibromochloromethane or bromoform as part of a trihalomethane mixture was retained in the soft tissues of rats 8 hours after dosing. The tissues which contained measurable amounts of the radiolabel were the brain, kidney, liver, lungs, muscle, pancreas, stomach (excluding contents), thymus, and urinary bladder. The relative amount of radiolabel in each tissue was not mentioned. Similar results were noted in mice, except that blood also contained a significant fraction of the total dose (10% in the case of bromoform). The chemical form of the material in the tissues (parent, metabolite, or adduct) was not reported. The form in blood also was not determined, but studies by Anders et al. (1978) suggest that some or all may have been carbon monoxide bound to hemoglobin (see Section 3.4.3).

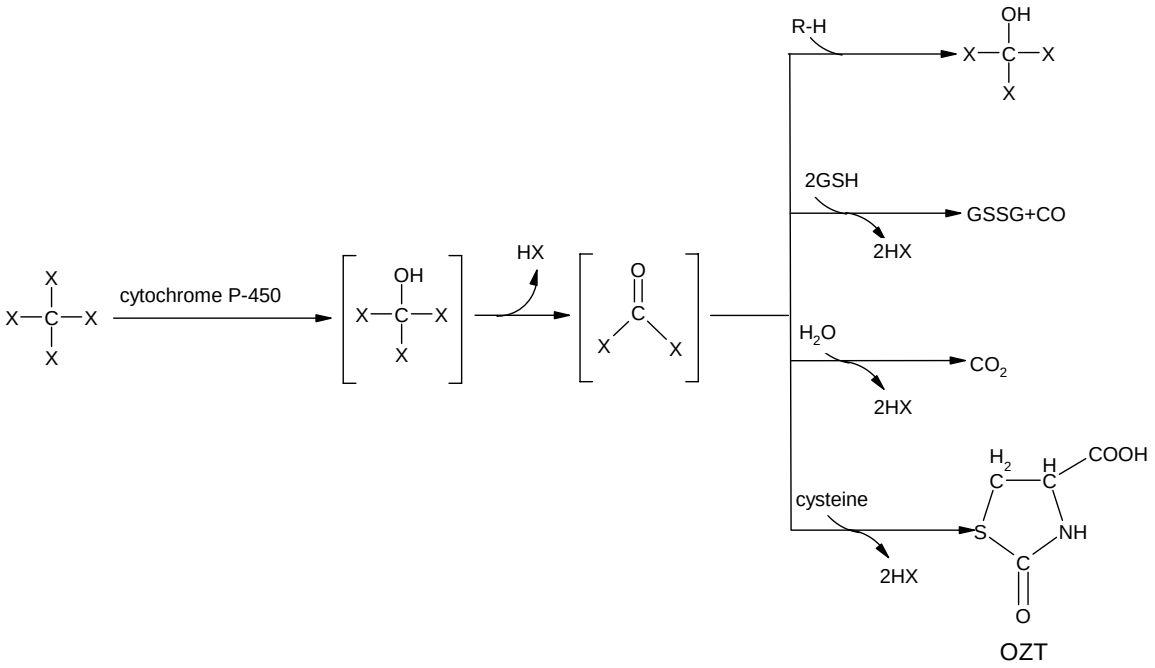
3.4.2.3 Dermal Exposure

No studies were located regarding the distribution of bromoform or dibromochloromethane in humans or animals following dermal exposure.

3.4.3 Metabolism

The metabolism of bromoform, dibromochloromethane, and other trihalomethanes has been investigated by Anders and colleagues (Ahmed et al. 1977; Anders et al. 1978; Stevens and Anders 1979, 1981). The main reactions, which are not believed to be route-dependent, are shown in Figure 3-3. The first step in the metabolism of trihalomethanes is oxidation by the cytochrome P-450 mixed function oxidase system of liver. This has been demonstrated *in vitro* using isolated rat liver microsomes (Ahmed et al. 1977), and *in vivo*, where the rate of metabolism is increased by cytochrome P-450 inducers (phenobarbital) and decreased by cytochrome P-450 inhibitors (SKF-525A) (Anders et al. 1978). The product of this reaction is presumed to be trihalomethanol, which then decomposes by loss of hydrogen and halide ions to yield the dihalocarbonyl. Although this intermediate has not been isolated, its formation has been inferred by the detection of 2-oxothiazolidine-4-carboxylic acid (OZT) in an *in vitro* microsomal system metabolizing bromoform in the presence of cysteine (Stevens and Anders 1979). The dihalocarbonyl molecule (an

Figure 3-3. Proposed Pathway of Trihalomethane Metabolism in Rats*



*Adapted from Stevens and Anders 1981

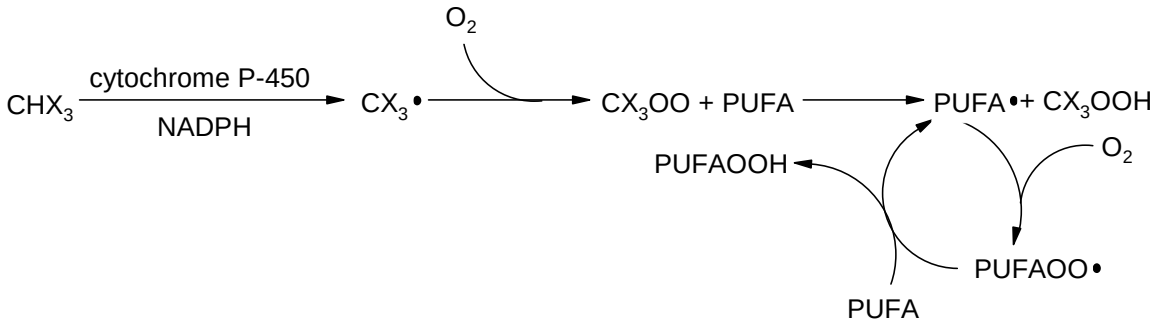
X = halogen atom (chlorine, bromine); R = cellular nucleophile (protein, nucleic acid); GSH = reduced glutathione; GSSG = oxidized glutathione; OZT = oxothiazolidine carboxylic acid

analogue of phosgene) is highly reactive, and may undergo a number of reactions, including: (a) direct reaction with cellular nucleophiles to yield covalent adducts; (b) reaction with two moles of glutathione (GSH) to yield carbon monoxide and oxidized glutathione (GSSG); and (c) hydrolysis to yield CO_2. The amount of trihalomethane metabolized by each of these pathways has not been studied in detail, but it appears that conversion to CO_2 is the main route. However, this depends on the species, the trihalomethane being metabolized, and metabolic conditions (cellular glutathione levels). Mink et al. (1986) found that mice oxidized 72% of an oral dose of dibromochloromethane and 40% of an oral dose of bromoform to CO_2. In contrast, rats oxidized only 18% of dibromochloromethane and 4% of bromoform to CO_2. The fraction of the dose converted to carbon monoxide has not been quantified, but dramatically increased levels of carboxyhemoglobin have been reported following oral exposure of rats to bromoform (Anders et al. 1978; Stevens and Anders 1981). Mink et al. (1986) reported that about 10% of a dose of bromoform was present in blood in mice; the form of the label was not investigated, but it may have been carboxyhemoglobin.

Metabolism of trihalomethanes by cytochrome P-450 can also lead to the production of highly reactive trihalomethyl free radicals, especially under hypoxic conditions (O'Brien 1988). Radical formation from bromoform has been observed both in isolated hepatocytes incubated with bromoform *in vitro* and in the liver of rats exposed to bromoform *in vivo* (Tomasi et al. 1985). Although it has not been studied, it seems likely that this pathway would also generate trihalomethyl radicals from dibromochloromethane. While metabolism to free radicals is a minor pathway in the sense that only a small fraction of the total dose is converted, it might be an important component of the toxic and carcinogenic mechanism of dibromochloromethane and bromoform. Figure 3-4 shows how free radical generation can lead to an autocatalytic peroxidation of polyunsaturated fatty acids (PLJFAs) in cellular phospholipids (O'Brien 1988). Peroxidation of cellular lipids has been observed in rat kidney slices incubated with bromoform *in vitro*, although lipid peroxidation was not detectable in liver slices (Fraga et al. 1987). Lipid peroxidation is considered to be a likely cause of cell injury for other halogenated compounds such as carbon tetrachloride (Agency for Toxicological Substances and Disease Registry 1994), but the significance of this pathway in the toxicity of dibromochloromethane and bromoform remains to be determined.

Development of the general metabolic scheme for trihalomethanes shown in Figure 3-3 relied primarily on the use of chloroform and bromoform as model substrates. However, some metabolic data specific to dibromochloromethane are available from a series of experiments conducted in rats by Pankow et al. (1997). Increased levels of the metabolites bromide (in plasma) and carbon monoxide (as detected by

Figure 3-4. Proposed Pathway of Trihalomethyl-radical-mediated Lipid Peroxidation*



*Adapted from O'Brien 1988

X = halogen atom (chlorine, bromine, iodine); PUFA = polyunsaturated fatty acid

formation of carboxyhemoglobin in blood) and dependence of their rate of production on hepatic GSH concentration in rats treated with dibromochloromethane are consistent with the metabolic scheme for the oxidative pathway shown in Figure 3-3. Observation of increased hepatic levels of GSSG in dosed rats by Pankow et al. (1997) is also consistent with the proposed scheme. The observation of (1) partial inhibition of bromide and carboxyhemoglobin production in dosed rats co-administered diethyldithio-carbamate (a potent inhibitor of P-450 isoform CYP2E1) and (2) stimulation of production in rats pretreated with isoniazid (a potent inducer of CYP2E1) indicates that cytochrome P-450 isoform CYP2E1 is at least partially responsible for oxidative metabolism of dibromochloromethane. Pretreatment with phenobarbital (an inducer of CYP2B1/2 in the rat) increased the concentration of bromide in plasma, suggesting that CYP2B1/2, as well as CYP2E1, catalyzes metabolism of dibromochloromethane in the rat.

Recent studies suggest that brominated trihalomethanes, including bromoform and dibromochloro-methane, may be metabolized by one or more pathways dependent on glutathione conjugation catalyzed by glutathione S-transferase (De Marini et al. 1997; Pegram et al. 1977) in addition to the oxidative and reductive pathways described above. Transfection of *Salmonella typhimurium* test strain TA1535 with the gene for rat glutathione S-transferase theta 1-1 increased the mutagenicity of bromoform and dibromochloromethane (by 95- and 85-fold, respectively) in reverse mutation assays when compared to mutagencity in TA1535 transfected with a nonfunctional form of the gene (De Marini et al. 1997). The mutational spectra and site specificity for bromoform, dibromochloromethane, and bromodichloro-methane (a structurally-related brominated trihalomethane) were closely similar, suggesting bioactivation of all three compounds via common metabolic intermediates. The mutagenic metabolites were not identified in this study, but S-(1-halomethyl)glutathione, S-(1,1-dihalomethyl) glutathione, or their further metabolites were presumed on the basis of data reported for other halomethanes to be capable of reacting with DNA to produce the observed mutations (De Marini et al. 1997).

The formation of reactive metabolites by conjugation of bromoform or dibromochloromethane via glutathione S-transferase theta-mediated pathways has not been examined in mammalian test systems. However, the metabolism of bromodichloromethane via glutathione conjugation has been investigated *in vitro* using cytosols prepared from human, rat, and mouse liver (Ross and Pegram 2003). Conjugation of bromodichloromethane with glutathione in mouse liver cytosol was time- and protein-dependent, was not affected by an inhibitor of alpha-, mu-, and pi-class glutathione S-transferases, and was correlated with activity toward 1,2-epoxy-3-(4'-nitrophenoxy)propane (a substrate specific for theta-class glutathione S-transferases). Conjugation activity toward bromodichloromethane in hepatic cytosols isolated from

different species followed the rank order mouse, followed by rat, then human. The initial conjugate formed was S-chloromethyl glutathione. This compound was unstable and degraded to multiple metabolites including S-hydroxymethyl glutathione, S-formyl glutathione, and formic acid. These data demonstrate that glutathione conjugation of bromodichloromethane occurs in mammalian liver cytosols and is likely catalyzed by glutathione S-transferase theta. These findings are significant because production of reactive glutathione conjugates from bromodichloromethane may result in formation of DNA adducts and thus cause genotoxicity. Because there is structural similarity among the brominated trihalomethanes and evidence for common pathways of bioactivation (De Marini et al. 1997), the findings of Ross and Pegram (2003) support the idea that glutathione conjugation of bromoform and dibromochloromethane leading to formation of reactive metabolites also occurs in the liver of rodents and humans.

Comparison of catalytic efficiencies for recombinant rat CYP2E1 and glutathione S-transferase theta 1-1 using bromodichloromethane as a substrate (data on bromoform or dibromochloromethane are not available) suggest that glutathione conjugation is likely to be a quantitatively minor hepatic pathway *in vivo* (Ross and Pegram 2003). Although metabolism via this pathway may be minor from the standpoint that only a small fraction of the total dose is converted, it might be an important component of the mechanism for dibromochloromethane and/or bromoform toxicity in extrahepatic tissues where GSTT1-1 is expressed in conjunction with lower levels of CYP2E1.

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

No studies were located regarding excretion of bromoform or dibromochloromethane by humans or animals following inhalation exposure.

3.4.4.2 Oral Exposure

In rats and mice given a single oral dose of ^{14}C-labeled bromoform or dibromochloromethane as part of a trihalomethane mixture (100 mg/kg in rats and 150 mg/kg in mice), excretion occurred primarily by exhalation of bromoform or dibromochloromethane or of CO_2 (Mink et al. 1986). In mice, 39.68 and 71.58% of the administered radiolabelled bromoform and dibromochloromethane, respectively, were exhaled as CO_2 and 7.18 and 12.31% as unmetabolized compound, respectively. In contrast, 4.3 and

18.2% of bromoform and dibromochloromethane were exhaled as CO_2 in rats and 66.9 and 48.1% of parent compound. Only 1–5% of the dose was excreted in urine (the chemical form in urine was not determined). The elimination half-times of bromoform and dibromochloromethane were 0.8 and 1.2 hours, respectively, in rats at 8 or 2.5 hours, respectively, in mice.

3.4.4.3 Dermal Exposure

No studies were located regarding excretion of bromoform or dibromochloromethane by humans or animals following dermal exposure.

3.4.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen et al. 1987; Andersen and Krishnan 1994). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parametrization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of

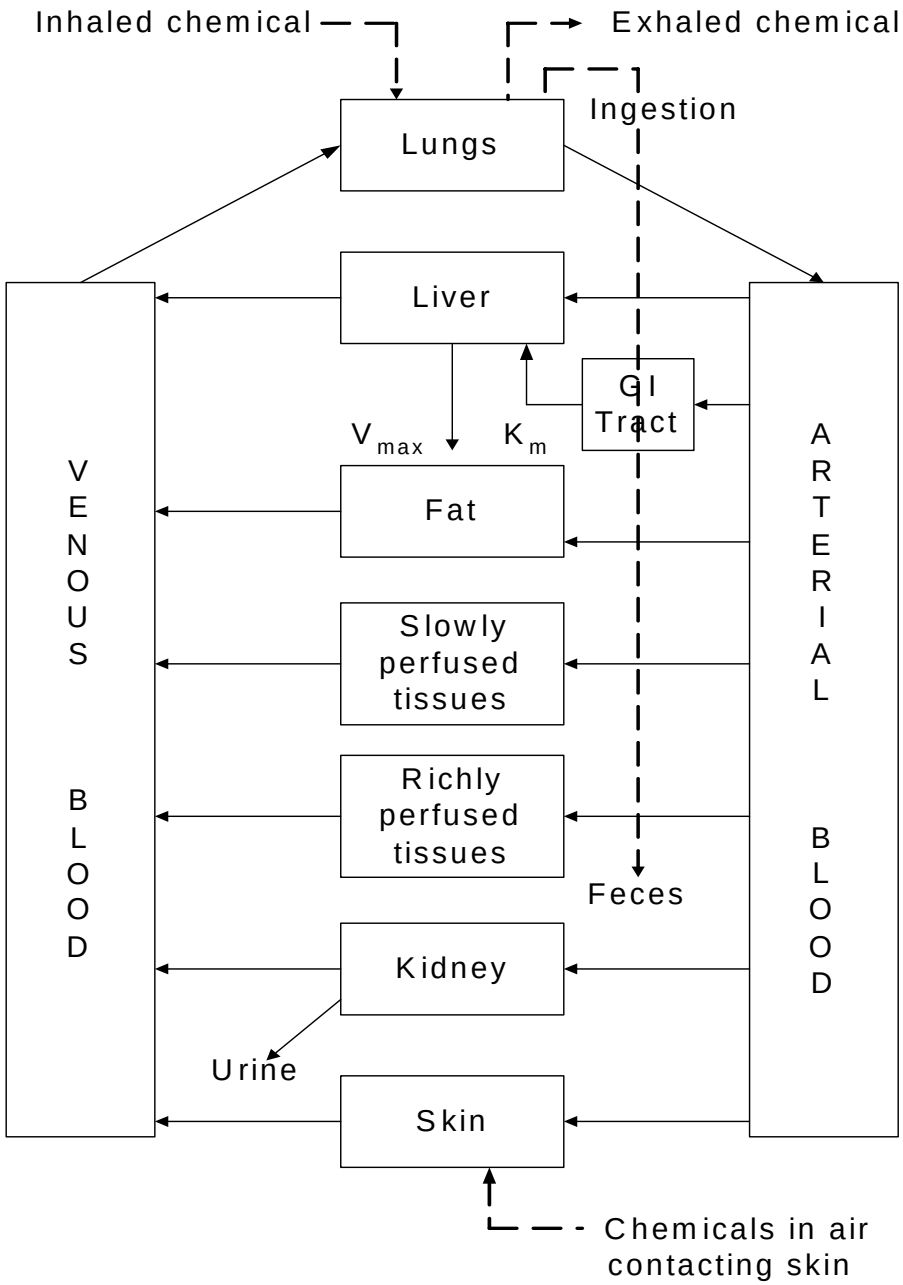
toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) are adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-5 shows a conceptualized representation of a PBPK model.

PBPK models have not been developed for bromoform or dibromochloromethane. da Silva et al. (1999) developed a model to simulate the venous blood concentration of each trihalomethane after simultaneous exposure to the four trihalomethanes. This model, which simulates competitive inhibition of hepatic metabolism, is discussed in Section 3.9. Although da Silva et al. (1999) also described models in rats following single administration of bromoform or dibromochloromethane, these models involved optimizing metabolic parameters across the dose range used in the study and were not developed as stand-alone models.

Figure 3-5. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Source: adapted from Krishnan et al. 1994

Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Bromoform and dibromochloromethane can be absorbed by inhalation (Graham 1915; Merzbach 1928), ingestion (Backer et al. 2000; Mink et al. 1986), and dermal (Backer et al. 2000; Xu et al. 2002) routes of exposure. In humans and laboratory animals, bromoform and dibromochloromethane are generally absorbed quickly (Backer et al. 2000). Although bromoform and dibromochloromethane are lipophilic, they do not appear to accumulate in adipose tissue (Stanley 1986). Bromoform and dibromochloromethane are thought to be metabolized by at least two route-independent pathways: oxidation by cytochrome P-450 mixed function oxidase system (Ahmed et al. 1977; Anders et al. 1978) and conjugation via glutathione S-transferase (DeMarini et al. 1997; Pegram et al. 1977). Bromoform and dibromochloromethane are primarily excreted via exhalation as the parent compound or carbon dioxide (Mink et al. 1986).

3.5.2 Mechanisms of Toxicity

The mechanisms by which bromoform and dibromochloromethane cause damage in target tissues is not fully understood, but there is strong evidence that metabolism to reactive intermediates is a prerequisite for toxicity. Subsequent interaction of the reactive intermediates with key cellular molecules leads to impaired function and/or cell death.

Several mechanisms for cell injury by trihalomethanes have been proposed based on the combined experimental database for chloroform and the brominated trihalomethanes. These include: (1) oxidative metabolism by CYP2E1 to produce dihalocarbonyls, which deplete glutathione content, and alkylate cellular macromolecules to produce necrosis; (2) reductive dehalogenation under conditions of low physiological oxygen tension to produce highly reactive dihalomethyl free radicals that covalently bind to proteins or lipids; and (3) glutathione-dependent metabolism to DNA-reactive intermediates, which results in adduct formation and mutation. Toxicity of bromoform and dibromochloromethane may result from metabolism by one or more of these pathways. Bromine is generally a better leaving group than chlorine, suggesting that the degree of bromine substitution will influence the rate of metabolism among chlorinated and brominated trihalomethanes and the flux through specific pathways.

Although mechanistic data on bromoform and dibromochloromethane are lacking, the requirement for metabolism of these compounds to toxic intermediates is supported by studies of chloroform and bromodichloromethane. In a representative study, Ilett et al. (1973) observed covalent binding of radiolabeled material to proteins in the liver and kidney following administration of [^{14}C]chloroform to mice. The amount of binding was correlated with the extent of renal and hepatic necrosis both in normal animals and in male mice pretreated with an inhibitor or inducer of microsomal enzymes. Autoradiograms showed that the bound radioactivity was located mainly in necrotic lesions. In other studies, Brown et al.(1974) reported that pretreatment of rats with phenobarbital (a cytochrome P-450 inducer) resulted in increased formation of covalent adducts and increased hepatic toxicity following chloroform exposure. Pohl et al. (1980) reported that the level of covalent binding correlated directly with injury to the liver tissue and concluded that phosgene was the metabolite responsible for the covalent binding to liver macromolecules. Tyson et al. (1983) confirmed that covalent binding to proteins in rats was more prevalent in areas of necrosis than in less damaged areas. More recently, Constan et al. (1999) evaluated toxicity in male B6C3F$_1$, SV/129 wild-type (CYP2E1 +/+), and SV/129 null (CYP2E1 -/-) mice exposed to chloroform by inhalation. Parallel groups of control and treated mice (B6C3F$_1$ and wild-type SV/129) were also treated with an irreversible cytochrome P-450 inhibitor. Extensive hepatic and renal necrosis occurred in B6C3F$_1$ and SV/129 mice exposed to chloroform. Chloroform-exposed animals that received the inhibitor were completely protected against hepatic and renal toxicity and pathological changes were absent in null mice, demonstrating that metabolism is necessary for toxicity.

The data linking metabolism of brominated trihalomethanes to toxicity are less extensive than those for chloroform. No data were located specifically for bromoform or dibromochloromethane. Pretreatment of female rats and mice with inhibitors of CYP2E1 metabolism reduced the acute renal and hepatic toxicity of bromodichloromethane (Thornton-Manning et al. 1994). Depletion of glutathione by pretreatment of male F344 rats with butathione sulfoximine (BSO) increased the incidence and severity of hepatic and renal lesions (Gao et al. 1996). Addition of glutathione to hepatic microsomal and S9 preparations and renal microsomes under aerobic conditions decreased covalent binding of [^{14}C]bromodichloromethane to proteins. Addition of glutathione to hepatic or renal microsomes under anaerobic conditions decreased binding of [^{14}C]bromodichloromethane to lipids (Gao et al. 1996). These data demonstrate a protective role of glutathione that is consistent with metabolism of bromodichloromethane to one or more reactive species.

Studies in animals indicate that bromoform and dibromochloromethane have carcinogenic potential (NTP 1985, 1989a). The mechanism of action for tumor induction by these chemicals is unknown and may

involve contributions from more than one of the proposed pathways described above. Pathway 1 (oxidative metabolism to dihalocarbonyl) may not be directly genotoxic, but may lead to increased risk of cancer as a result of cytotoxicity and subsequent regenerative hyperplasia, as observed for chloroform (ILSI 1997). Pathways 2 (reductive metabolism) and 3 (glutathione-dependent metabolism) may lead to direct DNA damage *in vivo*. The relative contribution of these pathways to the carcinogenic potential of bromoform and dibromochloromethane has not been determined.

Three studies have examined cytotoxicity and regenerative hyperplasia in the liver of female $B6C3F_1$ mice (Coffin et al. 2000; Melnick et al. 1998) or the kidney of male F344 rats (Potter et al. 1996) following exposure to bromoform or dibromochloromethane. These studies are of limited use for understanding the mechanism of bromoform carcinogenesis because they do not address cytotoxicity or regeneration in the large intestine, the target tissue for tumor induction. Dibromochloromethane induced cytotoxicity and cell proliferation in the liver of female $B6C3F_1$ mice, but the LOAEL values reported by Melnick et al. (1998) and Coffin et al. (2000) were inconsistent (400 vs. 100 mg/kg/day). Therefore, a threshold for induction of cellular proliferation cannot be clearly identified for comparison with the liver tumor incidence data in female $B6C3F_1$ mice (NTP 1985). The reason for the discrepancy between studies is unknown, but might be related to the use of different techniques or different time points for measurement of labeling index (a method for estimating cell proliferation). These data do not allow a conclusion regarding the role of cytotoxicity and regenerative cell proliferation in the development of hepatic tumors in mice exposed to dibromochloromethane. Melnick et al. (1998) modeled dose response data for cell proliferation and serum enzyme activity (as a composite) and for tumor induction in the liver of $B6C3F_1$ mice using an empirical Hill equation model. The dose-response curves for these processes had different shapes, suggesting that they were not causally associated.

Positive results for mutagenicity and cytogenetic damage have been observed in some standard assays (see Section 3.3), suggesting that some metabolites of bromoform and dibromochloromethane interact directly with DNA. Reductive metabolism (Melnick et al. 1998) and glutathione conjugation of brominated trihalomethanes have been proposed as a source of DNA reactive intermediates (De Marini et al. 1997; Ross and Pegram 2003), but the available data are too limited to fully evaluate the contribution of these pathways to the genotoxicity and carcinogenicity of bromoform and dibromochloromethane. Studies of bromodichloromethane metabolism in hepatic cytosols prepared from rat, mouse, and liver microsomes suggest that metabolism of brominated trihalomethanes via a glutathione-dependent pathway is likely to be a minor pathway in the liver (Ross and Pegram 2003). It is unknown whether the small amounts formed in the liver are toxicologically significant. It is possible that glutathione-dependent

pathways are of greater toxicological significance in extrahepatic tissues where concentrations of CYP2E1 are lower, such as the distal large intestine (Ross and Pegram 2003).

3.5.3 Animal-to-Human Extrapolations

Most of the available data on the toxicity and toxicokinetic properties of bromoform and dibromochloromethane come from studies in rats and mice. There are very limited human data, and the only end point that has been examined is neurotoxicity; high doses of bromoform result in central nervous system depression in humans and laboratory animals. Data from other trihalomethanes, particularly chloroform (Agency for Toxic Substances and Disease Registry 1997), suggest that the available laboratory animal toxicity and toxicokinetic data for bromoform and dibromochloromethane would also be applicable for humans. A potential difference between species is quantitative and qualitative differences in Phase I metabolism of trihalomethanes. In rats, trihalomethanes are substrates of cytochrome P-450 enzyme isoforms CYP2E1, CYP2B1, CY2B2, and CYP1A2 (Allis et al. 2001; Pankow et al. 1997). CYP2E1 and CPY1A2 are also expressed in human tissues; however, the relevance of metabolism by CYP2B1/2 to human health is presently uncertain, since these isoforms have not been reported in human adult or fetal tissues (Juchau et al. 1998; Nelson et al. 1996). CYP2B6 is the only active member of the CYP2B subfamily in man, although the CYP2B7 gene has also been found in the genome (Czerwinski et al. 1994).

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Colborn and Clement (1992), was also used in 1996 when Congress mandated the EPA to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), and in 1998, the EDSTAC completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulator*s has also been used to

convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

No *in vitro* studies were located regarding endocrine disruption of bromoform and dibromochloromethane.

There is little evidence to suggest that bromoform or dibromochloromethane has the potential to distrupt the normal functioning of the neuroendocrine axis. There are some suggestive data that these chemicals have the potential to alter reproductive hormone levels. An ecological study found significant correlations between bromoform and dibromochloromethane concentrations in drinking water and alterations in menstrual cycle length (Windham et al. 2003); however, the women were exposed to a number of other potentially toxic compounds in the drinking water and the data are inadequate for establishing causality. In rats administered 310–380 mg/kg/day bromoform or dibromochloromethane, significant decreases in serum testosterone levels were observed (Potter 1996).

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect

effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6, Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

There are limited data on the toxicity of bromoform and dibromochloromethane. In the early 1900s, bromoform was used as a sedative for the treatment of whooping cough (Benson 1907; Burton-Fanning 1901; Dwelle 1903; Stokes 1900; von Oettingen 1955). Sedation was observed at the prescribed dosage; higher doses often resulted in central nervous system depression. Central nervous system depression has also been observed in adult animals following oral exposure to bromoform (Bowman et al. 1978; NTP 1989a) or dibromochloromethane (Bowman et al. 1978; NTP 1985). There is no indication that children are more susceptible than adults to this effect.

Animal data provide strong evidence that the liver is the critical target of bromoform and dibromochloromethane toxicity (Aida et al. 1992; Coffin et al. 2000; Condie et al. 1983; NTP 1985, 1989a; Tobe et al. 1982). These animal studies were conducted in adults and no animal studies using juveniles were located. The available data suggest that the hepatotoxicity of bromoform and dibromochloromethane is due to tissue damage from reactive intermediates that are generated during metabolism. In rats, trihalomethanes are substrates for several cytochrome P-450 enzyme isoforms, including CYP2E1, CYP2B1, CYP2B2, and CYP1A2 (Allis et al. 2001; Pankow et al. 1997) in Phase I reactions and glutathione S-transferase theta (GSTT) (DeMarini et al. 1997; Pegram et al. 1987; Ross and Pegram 2003) in Phase II reactions. As discussed in EPA (2001a), CYP2E1 levels increase rapidly during the first 24 hours after birth and levels in children between 1 and 10 years of age are similar to those in adults. Similarly, GSTT levels in children older than 1 year are similar to adults. These data provide some suggestive evidence that exposure during early childhood would result in the formation of similar reactive intermediates and metabolites as in adults.

The potential of bromoform and dibromochloromethane to induce developmental effects cannot be conclusively established from the existing database. A significant alteration in the occurrence of sternebra aberrations were observed in the offspring of rats administered 200 mg/kg/day bromoform in corn oil during gestation (Ruddick et al. 1983). A similar exposure to dibromochloromethane did not result in any significant alterations. Similarly, no developmental alterations were observed in mouse

offspring exposed to 685 mg/kg/day dibromochloromethane in drinking water for several generations (Borzelleca and Carchman 1982).

There are no specific biomarkers of exposure or effect for bromoform or dibromochloromethane that have been validated in children or adults exposed as children. No studies were located regarding interactions of bromoform or dibromochloromethane with other chemicals in children. Additionally, there are no pediatric-specific methods of reducing peak absorption or reducing body burden following exposure to bromoform or dibromochloromethane. In the absence of these data, it is assumed that there are no age-related differences in biomarkers, interactions, or mitigation of effects.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to bromoform and dibromochloromethane are discussed in Section 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health

impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by bromoform and dibromochloromethane are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10 “Populations that are Unusually Susceptible.”

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Bromoform and Dibromochloromethane

The most straightforward means of identifying exposure to bromoform or dibromochloromethane in a person is measurement of parent compound in blood or expired air. Sensitive and specific gas chromatographic-mass spectrophotometric methods available for this purpose are described in Section 7.1. Quantification of exposure is complicated by the relatively rapid clearance rate of these compounds from the body, both by exhalation and metabolic breakdown. Data are not available on clearance rates in humans, but in animals, clearance of parent is nearly complete within 8 hours (see Section 3.4.4). Consequently, this approach is best suited for detecting recent exposures (within 1–2 days).

No data are available on blood or breath levels of bromoform or dibromochloromethane in acutely exposed individuals. However, studies involving exposure to trihalomethanes in water have found elevated alveolar air levels of dibromochloromethane following a 1-hour exposure to volatilized trihalomethanes from swimming pool water (Aggazzotti et al. 1998) and elevated blood bromoform and dibromochloromethane levels following a 10-minute shower or 10-minute bath (Backer et al. 2000). Background concentrations in people not exposed to bromoform or dibromochloromethane except through chlorinated drinking water are about 0.6 ppb (Antoine et al. 1986), while levels in expired breath are undetectable (Wallace et al. 1986a, 1986b). In a study of blood trihalomethane concentrations in women living in areas with trihalomethane in tap water (Miles et al. 2002), a simple linear relationship

between blood levels and tap water levels of trihalomethanes was not found. For bromoform and dibromochloromethane, no significant correlations were found between tap water levels of these compounds and blood levels before showering. After showering, a significant correlation between bromoform levels in the tap water and blood levels were found; however, the correlation coefficient (0.450) is relatively low, suggesting that a number of variables affected blood bromoform levels. Although bromoform and dibromochloromethane are lipophilic, they do not appear to accumulate in adipose tissue (Stanley 1986), so measurement of parent levels in this tissue is not likely to be valuable as a biomarker of exposure.

The principal metabolites of bromoform and dibromochloromethane are CO_2, CO, Cl^-, and Br^-. None of these metabolites are sufficiently specific to be useful as a biomarker of exposure. It is suspected that reactive intermediates formed during metabolism may produce covalent adducts with proteins or other cellular macromolecules (see Section 3.4.3), but these putative adducts have not been identified nor has any means for their quantification been developed.

3.8.2 Biomarkers Used to Characterize Effects Caused by Bromoform and Dibromochloromethane

There are limited data on the toxicity of bromoform and dibromochloromethane in humans. Exposure to bromoform can result in sedation; it is likely that the same is true for dibromochloromethane. However, generalized central nervous system depression is too nonspecific to be useful as a biomarker of effects from bromoform or dibromochloromethane exposure. Studies in animals indicate the liver and the kidneys are also affected, resulting in fatty liver, increased serum enzyme levels, and nephrosis. Effects on liver and kidney can be evaluated using a variety of laboratory and clinical tests (CDC/ATSDR 1990), but these are also too nonspecific to be valuable in recognizing early effects caused by low level exposure to these two chemicals.

3.9 INTERACTIONS WITH OTHER CHEMICALS

It is well-known that exposure to alcohols, ketones, and a variety of other substances can dramatically increase the acute toxicity of halomethanes such as carbon tetrachloride (Agency for Toxic Substances and Disease Registry 1994) and chloroform (Agency for Toxic Substances and Disease Registry 1997).

Several studies have been performed to determine if the toxic effects of bromoform and dibromochloromethane are similarly affected by these agents.

Hewitt et al. (1983) found that pretreatment of rats with a single oral dose of acetone resulted in a l0–40-fold potentiation of the hepatotoxic effects of a single oral dose of dibromochloromethane given 18 hours later. Similarly, pretreatment of rats for one to three days with chlordecone resulted in a 7–60-fold potentiation of the hepatotoxic effects of a single oral dose of dibromochloromethane (Plaa and Hewitt 1982a, 1982b). In contrast, chlordecone pretreatment had relatively little potentiating effect on the hepatotoxicity of bromoform (Agarwal and Mehendale 1983; Plaa and Hewitt 1982a).

The mechanism by which chemicals such as acetone and chlordecone potentiate halomethane toxicity is not known, but it is generally considered that at least some of the effect is due to stimulation of metabolic pathways that yield toxic intermediates. If so, the findings above support the hypothesis that the toxicity of dibromochloromethane is mediated at least in part by metabolic generation of reactive intermediates, but that metabolism is relatively less important in bromoform toxicity.

Harris et al. (1982) found that exposure of rats to a combination of bromoform and carbon tetrachloride resulted in more liver injury (judged by release of hepatic enzymes into serum) than would be predicted by the effects of each chemical acting alone. The mechanism of this interaction is not certain, but may be related to dihalocarbonyl formation and lipid peroxidation (Harris et al. 1982).

A PBPK model has been developed by da Silva et al. (1999) that examined the influence of exposure to a mixture of trihalomethanes (bromoform, dibromochloromethane, dichlorobromomethane, and chloroform) on the kinetics of individual compounds. When all four trihalomethanes were administered together (1.0 mmol/kg), the area under the blood concentration versus time curve was significantly higher as compared to the AUC after administration of a single compound (0.25 mmol/kg). For bromoform and dibromochloromethane, the AUCs after the single 0.25 mmol/kg dose were 48.6 and 31.2 μM/hour and after the 1.0 mmol/kg mixture of trihalomethanes, the AUCs were 127.3 and 128.6 μM/hour. These data suggest that there is metabolic interation between the trihalomethanes; likely competitive inhibition of hepatic metabolism.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to bromoform or dibromochloromethane than will most persons exposed to the same level of bromoform or dibromochloromethane in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of bromoform or dibromochloromethane, or compromised function of organs affected by bromoform or dibromochloromethane. Populations who are at greater risk due to their unusually high exposure to bromoform or dibromochloromethane are discussed in Section 6.7, Populations with Potentially High Exposures.

Studies of bromoform and dibromochloromethane toxicity in animals suggest that there may be some quantitative and qualitative differences in susceptibility between sexes and between species (see Section 3.2). The mechanistic basis for these differences is not known, but one likely factor is sex and species-dependent differences in metabolism (see Section 3.4.3). Thus, it is reasonable to assume that differences in susceptibility could exist between humans as a function of sex, age, or other metabolism-influencing factors. Studies in animals (discussed in Section 3.9) also suggest that humans exposed to alcohols, ketones, or other drugs (e.g., barbiturates, anticoagulants) that influence halomethane metabolism might be more susceptible to the toxic effect of dibromochloromethane and perhaps bromoform as well. Persons with existing renal or hepatic disease might also be more susceptible, since these organs are adversely affected by exposure to bromoform and dibromochloromethane. The elderly may represent an unusually suseptible population because they may have age-related deficiencies of liver and kidney function. They may also be frequently exposed to metabolism-influencing medications.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to bromoform or dibromochloromethane. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to bromoform or dibromochloromethane. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide specific information about treatment following exposures to bromoform or dibromochloromethane:

Haddad LM, Shannon MW, Winchester JF, eds. 1998. Clinimal management of poisoning and drug overdose. Philadelphia, PA: W.B. Saunders Co., 913-939, 992-1000.

Viccellio P, ed. 1998. Emergency toxicology. Philadelphia, PA: Lippincott-Raven Publishers, 925-932.

3.11.1 Reducing Peak Absorption Following Exposure

Human exposure to bromoform or dibromochloromethane may occur by inhalation, ingestion, or dermal contact. General recommendations for reducing absorption of these chemicals include removing the exposed individual from the contaminated area and removing the contaminated clothing. If the eyes and skin were exposed, they should be flushed with water. In order to reduce absorption of ingested bromoform and dibromochloromethane, emesis may be considered unless the patient is comatose, is convulsing, or has lost the gag reflex. Controversy exists concerning use of emesis because of the rapid onset of central nervous system depression, the risk of aspiration of stomach contents into the lungs, and the relative ineffectiveness of this method. In comatose patients with absent gag reflexes, an endotracheal intubation may be performed in advance to reduce the risk of aspiration pneumonia. Gastric lavage may also be used.

3.11.2 Reducing Body Burden

Trihalomethanes levels in human blood have a short half-life of approximately 0.5 hours (Lynberg et al. 2001). Bromoform or dibromochloromethane were not stored to any appreciable extent in the rat and were mostly metabolized to CO_2 (see Section 3.4). The elimination half-life of bromoform was reported to be 0.8 hours in the rat and 8 hours in the mouse (Mink et al. 1986). The half-lives of dibromochloromethane were 1.2 and 2.5 hours in rats and mice, respectively (Mink et al. 1986). Despite an expected relatively fast clearance of from the body, toxic effects may develop in exposed individuals. No method is commonly practiced to enhance the elimination of the absorbed dose of bromoform or dibromochloromethane.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

The primary targets of bromoform and dibromochloromethane are the liver, kidneys, and central nervous system. It is believed that the mechanism of bromoform and dibromochloromethane toxicity to the liver and kidneys involves metabolism to reactive intermediates. No methods for interfering with the

mechanism of action have been developed for these compounds. Studies using two other trihalomethanes (chloroform and dichlorobromomethane) provide suggestive evidence that administration of agents which inhibit cytochrome P-450 decreases hepatotoxicity. Co-administration of chloroform and 1-aminobenzo-triazole (an irreversible cytochrome P-450 inhibitor) (Constan et al. 1999) or dibromochloromethane and a CYP2E1 inhibitor (Thornton-Manning et al. 1994) resulted a reduction of acute hepatic and renal toxicity.

3.12 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of bromoform or dibromochloromethane is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of bromoform or dibromochloromethane.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

3.12.1 Existing Information on Health Effects of Bromoform and Dibromochloromethane

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to bromoform and dibromochloromethane are summarized in Figures 3-6 and 3-7, respectively. The purpose of this figure is to illustrate the existing information concerning the health effects of bromoform or dibromochloromethane. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a “data need”. A data need, as defined in ATSDR’s Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles (Agency for Toxic Substances and Disease Registry 1989), is substance-specific

Figure 3-6. Existing Information on Health Effects of Bromoform

	Death	Systemic: Acute	Systemic: Intermediate	Systemic: Chronic	Systemic: Immunologic/Lymphoretic	Systemic: Neurologic	Systemic: Reproductive	Systemic: Developmental	Systemic: Genotoxic	Cancer
Inhalation										
Oral	●					●				
Dermal										

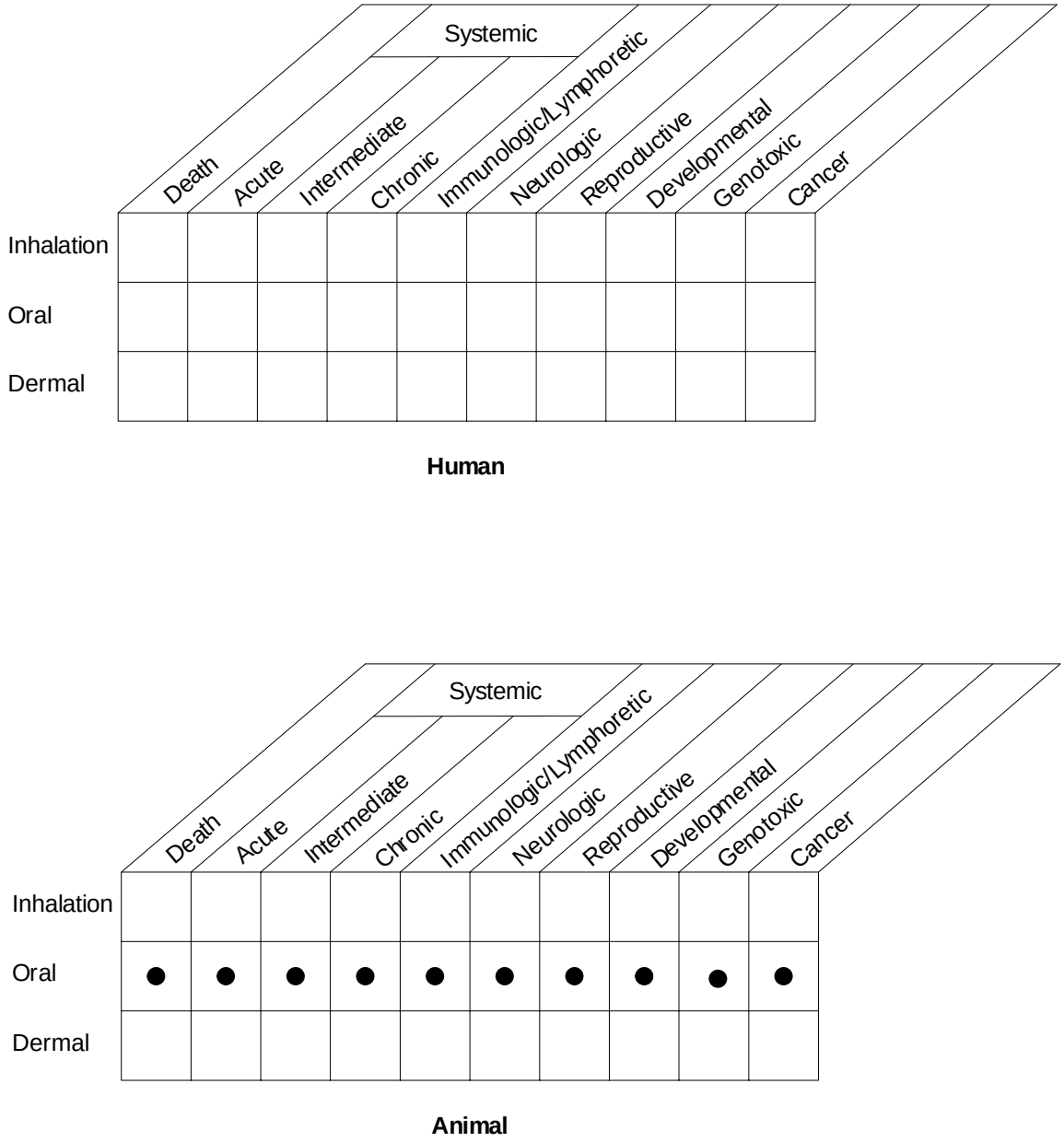
Human

	Death	Systemic: Acute	Systemic: Intermediate	Systemic: Chronic	Systemic: Immunologic/Lymphoretic	Systemic: Neurologic	Systemic: Reproductive	Systemic: Developmental	Systemic: Genotoxic	Cancer
Inhalation	●	●	●			●				
Oral	●	●	●	●	●	●	●	●	●	●
Dermal										

Animal

● Existing Studies

Figure 3-7. Existing Information on Health Effects of Dibromochloromethane



● Existing Studies

information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

As shown in Figure 3-6, there are limited data on the toxicity of bromoform following inhalation exposure. No human data were located and laboratory animal studies are limited to lethality studies, which found central nervous system depression, and acute and intermediate duration studies, which are only available as abstracts. Human data on the oral toxicity of bromoform are limited to reports of children taking bromoform for the treatment of whooping cough; effects are limited to death and central nervous system depression and the exposure levels are poorly characterized. A number of laboratory animal studies have examined the oral toxicity of bromoform, and data are available for all end points. However, in many cases, the available data for a particular end point are limited to a single study or use a route of administration (e.g., gavage) that may not be relevant to human environmental exposure to bromoform. The dermal toxicity of bromoform has not been studied in humans or animals.

As shown in Figure 3-7, there are no human toxicity data for dibromochloromethane following inhalation, oral, or dermal exposure. Additionally, there are no laboratory animal data on dibromochloromethane following inhalation or dermal exposure. For the oral route of exposure, data are available for all end points. As noted for bromoform, information on a particular end point comes from a single study, and most studies used a gavage in oil administration route, which may not be relevant for human environmental exposure to dibromochloromethane.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. The available data on the acute toxicity of bromoform in humans are limited to reports of children prescribed bromoform for the treatment of whooping cough. Following ingestion, the children were said to sleep undisturbed through the night, suggestive of sedation (Dwelle 1903); accidental overdoses resulted in symptoms of central nervous system depression (Benson 1907; Burton-Fanning 1901; Dwelle 1903; Stokes 1900; von Oettingen 1955). No human data were located discussing human toxicity following inhalation or dermal exposure to bromoform or after inhalation, oral, or dermal exposure to dibromochloromethane. Inhalation and oral exposure animal studies confirm that high oral doses of bromoform (Balster and Borzelleca 1982; Bowman et al. 1978; Graham 1915; Mertzbach 1928; NTP 1989a) or dibromochloromethane (Balster and Borzelleca 1982; NTP 1985) can result in central nervous system depression. At lower doses, the liver and possibly the kidney appear to be the primary targets of toxicity for both compounds. Nonlethal animal inhalation data are limited to a

study published in Russian that found liver and kidney effects in rats (Dykan 1964). The inhalation data are inadequate to define the threshold for these effects or identify the critical target of toxicity; additional inhalation studies that examine a wide range of end points and exposure concentrations would be useful for establishing the critical targets of toxicity of bromoform and dibromochloromethane following inhalation exposure and establishing concentration-response relationships. There is a more extensive database following oral exposure, which allows for the derivation of acute-duration oral MRLs for bromoform and dibromochloromethane. Following oral exposure to bromoform, increases in liver weight (Munson et al. 1982), centrilobular pallor (Condie et al. 1983), and hepatocellular vacuolization and/or swelling (Coffin et al. 2000) have been observed. Other effects of bromoform observed in acute-duration animal studies include mesangial nephrosis (Condie et al. 1983), impaired humoral immune function (Munson et al. 1982), and developmental effects (Ruddick et al. 1983). As with bromoform, the most sensitive effects of dibromochloromethane following acute oral exposure are liver and kidney damage; the liver effects consisted of mottled liver, hepatocellular vacuolization and/or swelling, and increases in SGPT and SGOT levels (Coffin et al. 2000; Condie et al. 1983; Munson et al. 1982; NTP 1985) and kidney effects included mesangial hyperplasia and darkened renal medullae (Condie et al. 1983; NTP 1985). At higher doses, impaired humoral immunity (Munson et al. 1982), decreased body weight gain (NTP 1985; Potter et al. 1996), and decreased serum testosterone levels (Potter et al. 1996) were observed.

No data are available in humans or animals following dermal exposure to bromoform or dibromochloromethane. Contact with concentrated solutions of these chemicals might be expected to produce effects similar to those following ingestion or inhalation, and might also result in skin or eye irritation. Studies on this would be useful, although contact with concentrated bromoform or dibromochloromethane is considered extremely unlikely for members of the general population or residents near waste sites. Studies on the effects of dermal contact with lower levels of the compounds in water or soil would be valuable, since people might be exposed by these routes near waste sites.

Intermediate-Duration Exposure. No human data are available on the intermediate-duration toxicity of bromoform or dibromochloromethane following inhalation, oral, or dermal exposure. Animal data are limited to a study reporting liver and kidney effects in rats exposed to bromoform for 2 months (Dykan 1962); however, this study is currently only available as an abstract. No intermediate-duration animal inhalation exposure data are available for dibromochloromethane. Further studies on the intermediate-duration inhalation toxicity of these compounds would be valuable in assessing human health risks from airborne exposures near waste sites, although available data suggest that exposures in air

near such sites are likely to be low. The available intermediate-duration oral exposure animal studies strongly identify the liver as the most sensitive target for bromoform and dibromochloromethane. Hepatocellular vacuolization and/or swelling have been observed at the lowest adverse effect levels (Aida et al. 1992; Daniel et al. 1990; NTP 1985, 1989a). Other effects observed in animals exposed to higher doses of bromoform include lethargy (NTP 1989a), impaired performance on neurobehavioral tests (Balster and Borzelleca 1982), and decreased body weight gain (Aida et al. 1992). Exposure to higher doses of dibromochloromethane also resulted in kidney damage (tubular degeneration and nephropathy) (Daniel et al. 1990; NTP 1985), impaired performance on neurobehavioral tests (Balster and Borzelleca 1982), and impaired fertility (Borzelleca and Carchman 1982). These animal data were considered adequate for derivation of an intermediate MRL for bromoform. An MRL was not derived for dibromochloromethane because the resultant MRL would be higher than the MRL for acute duration exposure. Additional studies using a number of dose levels would provide additional information on the threshold of hepatic toxicity and possible allow for the derivation of an intermediate-duration MRL for dibromochloromethane. There are no data on dermal exposure, and studies on intermediate-duration dermal exposure to the compounds in water or soil would be useful in evaluating human health risk at waste sites

Chronic-Duration Exposure and Cancer. No human studies examining the chronic toxicity of bromoform or dibromochloromethane were located, although a number of studies examining health outcomes in areas with elevated trihalomethanes in tap water have been located. Chronic animal inhalation data are not available for either chemical, and would be useful, especially for dibromochloromethane, since it is significantly more volatile (vapor pressure=76 mmHg) than bromoform (vapor pressure=5 mmHg). Chronic-duration studies that identify the critical targets of toxicity and establish concentration-response relationships would be useful for deriving chronic-duration inhalation MRLs for bromoform and dibromochloromethane. In the absence of such data, extrapolation of observations from the oral route might be possible using appropriate toxicokinetic models. The chronic oral toxicity of bromoform (NTP 1989a; Tobe et al. 1982) and dibromochloromethane (NTP 1985; Tobe et al. 1982) has been investigated in several studies, and the data are sufficient to identify hepatotoxicity as the most sensitive end point and to derive MRL values for both chemicals. However, in both cases, chronic oral MRLs are based on LOAELs for hepatotoxicity, so further studies to define the NOAELs would be helpful in reducing uncertainty in the MRL calculations. No data exist for dermal exposure, and further studies (focusing on exposure in water or soil) would be valuable.

The carcinogenic effects of chronic oral exposure to bromoform (NTP 1989a) and dibromochloromethane (NTP 1985) have been investigated in well designed studies in both rats and mice, and the data suggest that both chemicals have carcinogenic potential. However, effects were limited or equivocal in some cases, so additional studies to strengthen the weight of evidence would be valuable. Of particular interest would be studies of the carcinogenic effects when exposure is via drinking water rather than by gavage, since drinking water is the most likely route of human exposure, and exposure by gavage (especially using oil as a medium) may not be a good model for this. Also of value would be studies on the mechanism of carcinogenicity and on the identity of carcinogenic metabolites. For example, studies on methylene chloride and other volatile halocarbons indicate that metabolism via a glutathione pathway may be important in carcinogenicity (e.g., Anderson et al. 1987; Reitz et al. 1989). Studies to determine if dibromochloromethane or bromoform are metabolized by a similar pathway would be helpful in evaluating carcinogenic mechanism and risk.

Genotoxicity. There have been a number of studies that indicate bromoform and dibromochloromethane are genotoxic, both in prokaryotic (LeCurieux et al. 1995; Roldan-Arjona and Pueyo 1993; Simmon and Tardiff 1978; Simmon et al. 1977; Varma et al. 1988) and eukaryotic (Benigni et al. 1993; Fujie et al. 1993; Landi et al. 1999; Matsuoka et al. 1996; McGregor et al. 1991; Morimoto and Koizumi 1983; Nestman and Lee 1985; Sobti 1984; Sofuni et al. 1996) organisms. However, a number of other studies have failed to detect significant genotoxic potential for these compounds (Maddock and Kelly 1980; NTP 1985, 1989a). The basis for this inconsistency is not entirely obvious, but might be related to the efficacy of the test system to activate the parent compound to genotoxic metabolites. Several *in vivo* studies have also found genotoxic effects following exposure to bromoform (Fujie et al. 1990; Morimoto and Koizumi 1983; NTP 1989a) or dibromochloromethane (Fujie et al. 1990; Morimoto and Koizumi 1983), although other studies have not found effects (Hayashi et al. 1988; Potter et al. 1996; Stocker et al. 1996). Further studies to define conditions under which these compounds are and are not genotoxic *in vitro* and *in vivo* may help clarify both the mechanism of genotoxicity and the relevance of this to human health risk. Studies on the genotoxic effects of bromoform and dibromochloromethane on germ cells (sperm or ova) would also be valuable.

Reproductive Toxicity. A number of ecological studies have examined the reproductive toxicity to women ingesting drinking water contaminated with trihalomethanes (Bove et al. 1992, 1995; Dodds et al. 1999; Kramer et al. 1992; Mills et al. 1998; Nieuwenhuijsen et al. 2000; Savitz et al. 1995; Waller et al. 1999). Three of these studies (Kramer et al. 1992; Waller et al. 1999; Windham et al. 2003) measured bromoform and dibromochloromethane levels; however, the contribution of the other trihalomethanes to

the observed effects cannot be determined from the existing data. Collectively, the trihalomethane studies provide insufficient evidence to establish a causal relationship between exposure to trihalomethanes and adverse reproductive outcome. Animal data are limited to several studies that examined reproductive tissues for histological alterations or examined reproductive function following oral exposure to either compound. No histological alterations were observed in reproductive tissues following chronic exposure to bromoform (NTP 1989a) or dibromochloromethane (NTP 1985). No significant alterations in reproductive performance or fertility were observed in males or females exposed to bromoform (NTP 1989b). For dibromochloromethane, one study found a marked reduction in fertility at a maternally toxic dose level (Borzelleca and Carchman 1982), but no effects on reproductive performance or fertility were observed at lower doses (NTP 1986). Multigeneration studies involving oral exposure to bromoform and dibromochloromethane would provide useful data on the reproductive potential of these chemicals. Based on the oral studies, it does not seem likely that effects would occur following inhalation or dermal exposure except at very high levels, but inhalation and dermal exposure studies to confirm this important point would be valuable.

Developmental Toxicity. Several ecological studies have examined the relationship between exposure to trihalomethanes in tap water and the birth outcomes (Dodds et al. 1999; Kramer et al. 1992; Savitz et al. 1995). The Kramer et al. (1992) study reported levels for individual trihalomethanes, but this study, as well as the other studies, does not provide sufficient evidence for establishing a relationship between trihalomethane exposure and adverse birth outcomes. No other human studies were located for bromoform or dibromochloromethane. No inhalation or dermal exposure animal developmental toxicity studies were located. There are limited data on the developmental toxicity of bromoform and dibromochloromethane following oral exposure in animals. A minor skeletal abnormality was observed in the offspring of rats exposed to bromoform during gestation (Ruddick et al. 1983). No developmental effects were observed in studies of rat and mouse offspring exposed to dibromochloromethane (Borzelleca and Carchman 1982; Ruddick et al. 1983). Additional oral studies on the developmental effects of both bromoform and dibromochloromethane in animals would be valuable to determine whether these skeletal abnormalities are produced consistently, and whether they lead to significant adverse effects in the neonate. If so, then similar studies by the inhalation and dermal routes would also be valuable to define safe inhalation levels for developmental effects.

Immunotoxicity. The immunotoxic effects of bromoform and dibromochloromethane have been investigated in one 14-day oral study (Munson et al. 1982). That study indicated that both chemicals can lead to changes in several immune cell-types in mice. Similar studies in other species would be valuable

in determining if this is a common response. In addition, longer duration studies and tests of the functional consequence of these changes (e.g., resistance to infectious disease) would be especially valuable in assessing the biological significance of these effects. If these studies indicate the immune system is a target, then similar studies by inhalation and dermal exposure routes would also be valuable.

Neurotoxicity. Numerous studies, both in humans and animals, reveal that central nervous system depression is a rapid effect following either oral or inhalation exposure to bromoform; more limited data indicate that dibromochloromethane also causes this effect. While central nervous system depression appears to be reversible within a short time after exposure ceases, the possibility of permanent neurological damage from high doses has not been thoroughly studied. Histological studies by NTP indicate that sub-depressant doses of bromoform (NTP 1989a) and dibromochloromethane (NTP 1985) do not lead to detectable histological changes in the brain, but similar data are not available following narcotizing doses. In addition to histological studies, functional studies capable of detecting lasting neurological changes would be valuable. One study of this sort (Balster and Borzelleca 1982) indicates that both bromoform and dibromochloromethane can cause some behavioral changes (impaired motor performance, decreased exploratory behavior [bromoform only], and decreased response rate in tests of operant behavior) at high doses. Further studies along these lines, perhaps employing more sensitive tests of electrophysiological or neurobehavioral changes, would be helpful in determining if this is an effect of concern to exposed humans.

Epidemiological and Human Dosimetry Studies. No epidemiological or human dosimetry studies are currently available for bromoform or dibromochloromethane. Since only very small quantities of these chemicals are produced or used in this country (see Chapter 5), it does not seem likely that a sufficiently large subpopulation of exposed workers exists to serve as the basis for a meaningful epidemiological study. Epidemiological studies of populations exposed to low levels of bromoform and dibromochloromethane in chlorinated drinking water cannot provide specific data on the human health risks of dibromochloromethane or bromoform, since chlorinated drinking water contains hundreds of different contaminants.

Biomarkers of Exposure and Effect.

Exposure. The only known biomarker of exposure to bromoform or dibromochloromethane is the level of parent compound in blood or in expired air. However, data on blood or breath levels in humans following acute exposure are lacking, due to the rarity of such events. Since both bromoform and

dibromochloromethane are rapidly cleared from the body by exhalation or metabolism, measurements of parent compounds in blood or breath are likely to be useful only for a short time (1–2 days) after an exposure. Monitoring of humans continuously exposed to the trace levels normally present in chlorinated water reveal very low to nondetectable levels in blood or expired air. The main metabolites of these compounds (CO_2, CO, Cl^-, Br^-) are not sufficiently specific to be useful for biomonitoring of exposure. Identification of stable and specific biomarkers of exposure (e.g., halomethyl adducts) would be valuable in evaluating the exposure history of people around waste sites and other sources where above-average levels might be encountered.

Effect. No specific biomarkers of bromoform- or dibromochloromethane-induced effect are known. Neurological, hepatic, and renal effects caused by these chemicals can be detected by standard clinical or biochemical tests, but abnormal function in these tissues can be produced by a number of common diseases in humans, so detection of abnormal function is not proof that the effect was caused by bromoform or dibromochloromethane. Efforts to identify more specific and sensitive biomarkers of bromoform and dibromochloromethane-induced effects would be useful, especially biomarkers (e.g., specific DNA adducts) that might be predictive of carcinogenic risk.

Absorption, Distribution, Metabolism, Excretion. Limited data indicate that bromoform and dibromochloromethane are rapidly and efficiently taken up from the gastrointestinal tract, but further studies to confirm and refine available estimates would be valuable. Toxicokinetic studies to date have generally employed exposure by gavage in corn oil, so studies involving exposure via an aqueous vehicle would be especially valuable. No toxicokinetic data exist for inhalation exposure, so quantitative estimates of the inhalation absorption fraction, tissue distribution, and excretion rate would be beneficial. Also, data on dermal absorption would be helpful, especially from soil or from dilute aqueous solutions, since this is how humans are most likely to experience dermal contact near waste sites.

The pathways of bromoform and dibromochloromethane metabolism have been investigated in several laboratories, but quantitative data on the amount of chemical passing through each pathway are limited, and the chemical identity of products appearing in urine has not been studied. Of particular interest would be studies that seek to clarify the role of metabolism in toxicity, the mechanism by which metabolites and adducts lead to toxic effects, and the importance of protective mechanisms such as cellular antioxidants. This would include careful dose-response studies to determine if either activating or protective pathways are saturable.

Comparative Toxicokinetics. Available toxicity data indicate that target tissues of bromoform and dibromochloromethane are similar in humans, rats, and mice. Limited data suggest that effect levels are generally similar across species, but some distinctions are apparent. Toxicokinetic studies have revealed differences between rats and mice regarding metabolic patterns and clearance rates and these might underlie the differences in toxicity between tissues, sexes, and species. Additional comparative studies in animals, with special emphasis on differences in metabolism, would be useful in understanding these differences, and in improving inter-species extrapolation. In addition, *in vitro* studies of metabolism by human liver cells would be valuable in determining which animal species has the most similar pattern of metabolism and is the most appropriate model for human toxicity. Data from studies of this sort could then be used to support physiologically-based toxicokinetic models.

Methods for Reducing Toxic Effects. Further research is needed to determine strategies designed to selectively inhibit the specific P-450 isozymes involved in the metabolism of bromoform or dibromochloromethane to reactive intermediates, and thus reduce the toxic effects. Because bromoform or dibromochloromethane are thought to be metabolized by glutathione conjugation (DeMarini et al. 1997; Pegram et al. 1977; Ross and Pegram 2003), further research is needed to determine if administration of sulfhydryl compounds, such as L-cysteine, reduced GSH or N-acetylcysteine, as glutathione surrogates might provide protection against nephrotoxicity or hepatotoxicity induced due to depleted glutathione stores. Research on using dietary supplements for mitigating adverse effects of chronic exposure to bromoform or dibromochloromethane would be helpful, especially in the case of chronic exposure from chlorinated drinking water.

Children's Susceptibility. Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection above.

The available human data for bromoform are mostly from case reports of accidental orally-administered overdose in children. No data exist on the toxicity of dibromochloromethane following oral administration in children, or of bromoform or dibromochloromethane following inhalation or dermal exposure in children. The developmental effects of oral exposure to bromoform and dibromochloromethane have not been extensively investigated, but limited data suggest that these chemicals have relatively low toxicity on the developing fetus (Borzelleca and Carchman 1982; Ruddick et al. 1983). However, these studies did not examine neurodevelopmental end points that may be a sensitive target. Additional animal studies assessing the neurodevelopmental toxicity of bromoform and dibromochloro-

methane would be useful. Toxicokinetic studies examining how aging can influence absorption rates would be useful in assessing children's susceptibility to the toxicity of bromoform or dibromochloromethane.

Child health data needs relating to exposure are discussed in Section 6.8.1, Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

No ongoing studies on the health effects of bromoform or dibromochloromethane were listed in the Federal Research in Progress database (FEDRIP 2004).

4. CHEMICAL AND PHYSICAL INFORMATION

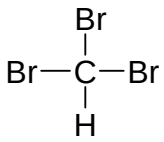
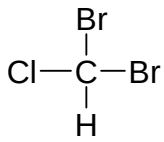
4.1 CHEMICAL IDENTITY

Information regarding the chemical identity of bromoform and dibromochloromethane is located in Table 4-1.

4.2 PHYSICAL AND CHEMICAL PROPERTIES

Information regarding the physical and chemical properties of bromoform and dibromochloromethane is located in Table 4-2.

Table 4-1. Chemical Identity of Bromoform and Dibromochloromethane

Characteristic	Bromoform	Dibromochloromethane	Reference
Synonyms	Tribromomethane; methenyltribromide; methane, tribromo-	Chlorodibromomethane dibromochloromethane; methane, dibromochloro-; methane, chlorodibromo-	HSDB 2004a, 2004b
Registered trade name(s)	No data	No data	
Chemical formula	$CHBr_3$	$CHBr_2Cl$	O'Neil et al. 2001
Chemical structure			
Identification numbers:			
CAS registry	75-25-2	124-48-1	O'Neil et al. 2001
NIOSH RTECS	PB5600000	PA6360000	RTECS 2003
EPA hazardous waste	U225	No data	RTECS 2003
OHM/TADS	No data	No data	HSDB 2004a, 2004b
DOT/UN/NA/IMCO shipping	UN2515; IMCO 6.1	No data	HSDB 2004a, 2004b
HSDB	2517	2763	HSDB 2004a, 2004b
NCI	C55130	C55254	RTECS 2003
Beilstein reference number	1731048	1731046	RTECS 2003
Beilstein handbook reference	4-01-00-00082	4-01-00-0081	RTECS 2003
Wisesser line notation	EYEE	GYEE	RTECS 2003

CAS = Chemical Abstracts Service; DOT/UN/NA/IMCO = Department of Transportation/United Nations/North America/International Maritime Dangerous Goods Code; EPA = Environmental Protection Agency; HSDB = Hazardous Substances Data Bank; NCI = National Cancer Institute; NIOSH = National Institute for Occupational Safety and Health; OHM/TADS = Oil and Hazardous Materials/Technical Assistance Data System; RTECS = Registry of Toxic Effects of Chemical Substances

Table 4-2. Physical and Chemical Properties of Bromoform and Dibromochloromethane

Property	Bromoform	Dibromochloromethane	Reference
Molecular weight	252.73	208.28	O'Neil et al. 2001
Color	Colorless	Colorless to pale yellow	Verschueren 2001
Physical state	Liquid	Liquid	Verschueren 2001
Melting point	8.0 °C	-20 °C	Lide 2000
Boiling point	149.1 °C	120 °C	Lide 2000
Density at 20 °C	2.899	2.451	Lide 2000
Odor	Sweet, similar to chloroform	No data	Verschueren 2001
Taste	Similar to chloroform	No data	Lewis 1997
Odor threshold:			
Water	0.51 mg/L	No data	Amoore and Hautala 1983
Air	13.45 mg/m^3	No data	Amoore and Hautala 1983
Solubility:			
Water	3.10x10^3 mg/L (25 °C)	2.7x10^3 mg/L (20 °C)	Horvath 1982; Heikes 1987
Organic solvents	Miscible in ethanol, benzene, petroleum ether, acetone, oils	Soluble in ethanol, ether, acetone	Lide 2000
Corrosivity	Will attack some forms of plastics, rubber, and coatings.	No data	HSDB 2004a
Partition coefficients:			
Log octanol/water	2.4	2.16	CITI 1992; Sangster 1994
Log K_{oc}	2.06	1.92	Mabey et al. 1982
Vapor pressure at 20 °C	5 mmHg	76 mmHg	Mabey et al. 1982
Vapor density	8.7 (air=1)	No data	IARC 1991a, 1991b
Henry's law constant	5.6x10^{-4} atm-m^3/mol	9.9x10^{-4} atm-m^3/mol	Mabey et al. 1982
Surface tension	41.53 dynes/cm	No data	Lewis 1997
Heat of vaporization	46.05 KJ/mol (25 °C)	No data	Lide 2000
Autoignition temperature	No data	No data	
Flashpoint	No data	No data	
Flammability limits	Non-flammable	Non-flammable	HSDB 2004a, 2004b
Conversion factor	1 ppm = 10.34 mg/m^3 1 mg/m^3 = 0.097 ppm	1 ppm = 8.52 mg/m^3 1 mg/m^3 = 0.12 ppm	IARC 1999a, 1999b

5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

5.1 PRODUCTION

Bromoform may be prepared from acetone and sodium hypobromite, by treating chloroform with aluminum bromide, or by electrolysis of potassium bromide in ethyl alcohol (HSDB 2004a; Stenger 1978).

Bromoform is currently produced by Geoliquids, Inc., Prospect Heights, Illinois and Sigma-Aldrich Fine Chemicals (SRI 2004). Bromoform was produced formerly by Dow Chemical Company, Midland, Michigan. In 1975, production of bromoform in the United States was estimated to be <500 metric tons (<1 million pounds) and the 1977 production was estimated at 50–500 metric tons (100,000–1 million pounds) (NTP 1989a; Orrell and Mackie 1988; Perwak et al. 1980). The production volume of bromoform reported by manufacturers in 1990, 1994, and 1998 was within the range of 10,000–500,000 pounds (5–230 metric tons) (IUR 2002). Production volume data were not listed for reporting year 2002.

Dibromochloromethane can be prepared by the addition of dibromochloroacetone to 5N sodium hydroxide (IARC 1991b). It can also be prepared by reaction of a mixture of chloroform and bromoform with triethylbenzylammonium chloride and sodium hydroxide (IARC 1991a). Available information indicates that dibromochloromethane is no longer produced commercially in the United States (SRI 2004).

Both bromoform and dibromochloromethane are inadvertently generated during water chlorination when chlorine reacts with endogenous organic materials such as humic and fulvic acid (Rook 1977). When chlorine is added to water, hypochlorous acid is formed (Wallace 1997). Hypochlorous acid reacts with humic or fulvic acids in the water to produce chloroform or it can oxidize any bromide ion that is present to form hypobromous acid, which leads to the formation of bromoform, dibromochloromethane, or bromodichloromethane. It is estimated that 17 kkg of bromoform and 204 kkg of dibromochloromethane were generated in this way in 1978 (Perwak et al. 1980). According to a model generated by Clark et al. (1996), higher bromide to chloride ratios promote the bromine substitution reaction over the chlorine substitution reaction with organic matter in chlorinated water. At higher bromide concentrations, levels of chloroform decreased, while levels of bromoform increased. Dibromochloromethane was found to

increase to a maximum level for bromide concentrations of 2.5 mg/L and then decline with increasing bromide concentration (Clark et al. 1996).

Table 5-1 summarizes information on companies that reported the production, import, or use of bromoform for the Toxics Release Inventory in 2002 (TRI02 2004). The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list.

5.2 IMPORT/EXPORT

Orrell and Mackie (1988) estimated that 6–9 kkg of bromoform were imported by Freeman Industries in the late 1980s. No current information was located on the import of bromoform and dibromochloromethane, but it is likely that little, if any, is imported.

5.3 USE

Bromoform is used in geological assaying as a heavy liquid for mineral ore separations based on differences in specific gravity (Verschueren 2001). It is used in the electronics industry in quality assurance programs (Orrell and Mackie 1988). Bromoform has been used as a catalyst, initiator, or sensitizer in polymer reactions and in the vulcanization of rubber (HSDB 2004a). As a solvent, bromoform has been used for waxes, greases, and oils as well as for liquid-solvent extractions and nuclear magnetic resonance (NMR) studies (Lewis 1997; NTP 1989a). Former uses of bromoform include a fire-resistant chemical ingredient, a gauge fluid ingredient, an intermediate in chemical syntheses, and a sedative and antitussive agent (HSDB 2004a; Perwak et al. 1980).

Dibromochloromethane is used in laboratory quantities only and there is no current commercial use for this compound (Perwak et al. 1980). Dibromochloromethane was used formerly as a chemical intermediate in the production of fire extinguishing agents, aerosol propellants, refrigerants, and pesticides (IARC 1991b).

5.4 DISPOSAL

Because bromoform and dibromochloromethane are listed as hazardous substances, land disposal of wastes containing these compounds is controlled by a number of federal regulations (see Chapter 8).

Table 5-1. Facilities that Produce, Process, or Use Bromoform

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
AR	5	0	999,999	1, 2, 4, 9, 12, 13
CA	1	100	999	12
LA	2	0	999	1, 5, 13
MS	1	0	99	12
NE	1	10,000	99,999	12
OH	1	1,000	9,999	12
TX	2	1,000	999,999	12

Source: TRI02 2004 (Data are from 2002)

[a]Post office state abbreviations used
[b]Amounts on site reported by facilities in each state
[c]Activities/Uses:

1. Produce
2. Import
3. Onsite use/processing
4. Sale/Distribution
5. Byproduct
6. Impurity
7. Reactant
8. Formulation Component
9. Article Component
10. Repackaging
11. Chemical Processing Aid
12. Manufacturing Aid
13. Ancillary/Other Uses
14. Process Impurity

Wastes containing dibromochloromethane or bromoform may be incinerated by rotary kiln, liquid injection, or fluidized bed methods. CERCLA reportable quantities for bromoform and dibromochloromethane released to the environment are those quantities ≥100 pounds or 45.4 kg.

The amount of bromoform and dibromochloromethane released or disposed of through industrial and/or laboratory use of these chemicals is not known, but is considered to be insignificant compared to the amount inadvertently generated by water chlorination processes (EPA 1987c; HSDB 2004a; Perwak et al. 1980).

6. POTENTIAL FOR HUMAN EXPOSURE

6.1 OVERVIEW

Bromoform has been identified in at least 140 sites while dibromochloromethane has been identified in at least 174 sites of the 1,662 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (HazDat 2005). However, the number of sites evaluated for bromoform and dibromochloromethane is not known. The frequency of these sites can be seen in Figures 6-1 and 6-2, respectively. Of these sites, 137 of the 140 bromoform sites are located within the United States, 1 is located in the Virgin Islands (not shown), and 2 are located in the Commonwealth of Puerto Rico (not shown). For dibromochloromethane, 172 of the 174 sites are located within the United States, 1 is located in the Virgin Islands, and 1 is located in the Commonwealth of Puerto Rico.

The principal route of human exposure to dibromochloromethane and bromoform is from the consumption of chlorinated drinking water. These chemicals are thought to form in the water as by-products from the reaction of chlorine with dissolved organic matter and bromide ions. Dibromochloromethane and bromoform concentrations in water are quite variable, but average levels are usually <5 μg/L.

Most dibromochloromethane and bromoform tend to volatilize from water when exposed to the air. The fate of these chemicals in air has not been investigated, but it is likely they are relatively stable, with half-lives of about 1–2 months. Most measurements of the concentration of these chemicals in air indicate that levels are quite low (<10 ppt).

Neither chemical is strongly adsorbed from water by soil materials, and it is likely that both readily migrate in groundwater. Neither chemical appears to be easily biodegradable under aerobic conditions, but they may readily biodegrade under anaerobic conditions.

6.2 RELEASES TO THE ENVIRONMENT

The TRI data should be used with caution because only certain types of facilities are required to report (EPA 1997). This is not an exhaustive list. Manufacturing and processing facilities are required to report

Figure 6-1. Frequency of NPL Sites with Bromoform Contamination

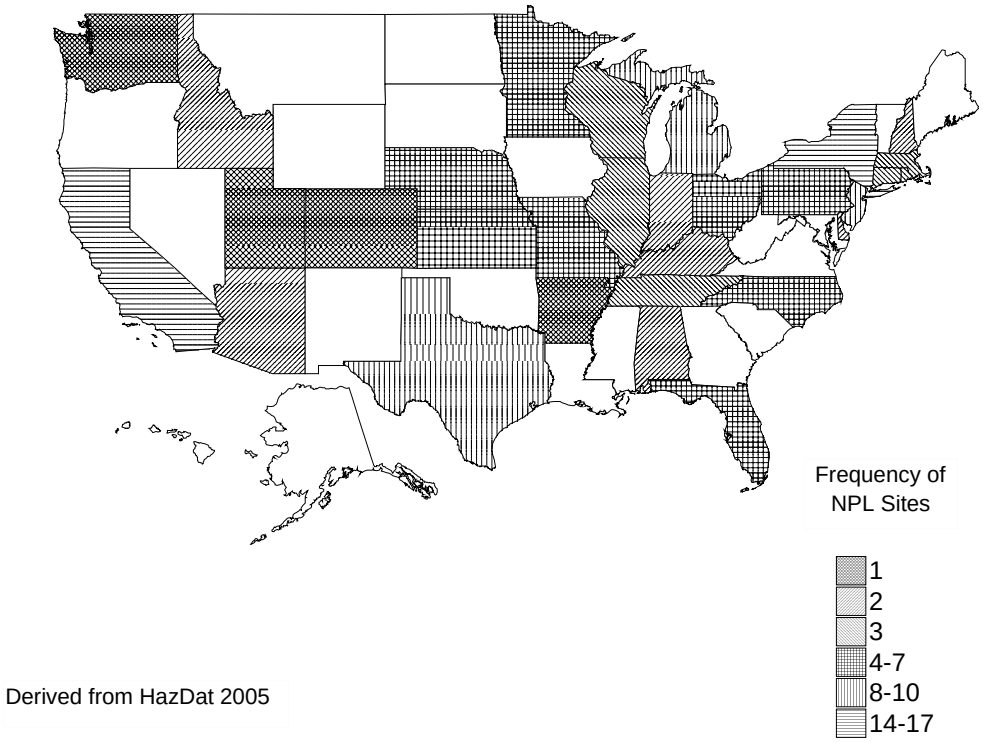
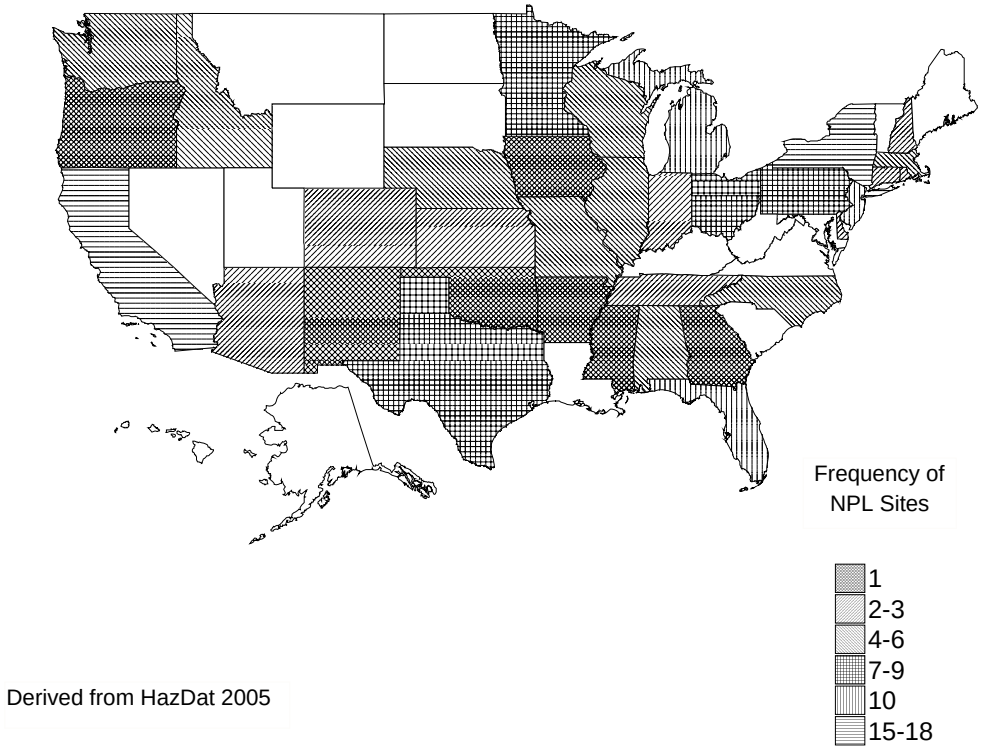


Figure 6-2. Frequency of NPL Sites with Dibromochloromethane Contamination



information to the Toxics Release Inventory only if they employ 10 or more full-time employees; if their facility is classified under Standard Industrial Classification (SIC) codes 20–39; and if their facility produces, imports, or processes ≥25,000 pounds of any TRI chemical or otherwise uses >10,000 pounds of a TRI chemical in a calendar year (EPA 1997).

Bromoform and dibromochloromethane have been identified in a variety of environmental media (air, surface water, groundwater, soil, and sediment) collected at 140 and 174 of the 1,662 NPL hazardous waste sites, respectively (HazDat 2005).

6.2.1 Air

Estimated releases of 12 pounds (0.01 metric tons) of bromoform to the atmosphere from 3 domestic manufacturing and processing facilities in 2002, accounted for about 3% of the estimated total environmental releases (TRI02 2004). These releases are summarized in Table 6-1.

Bromoform has been identified in air samples collected at 7 of the 140 NPL hazardous waste sites where it was detected in some environmental media (HazDat 2005). Dibromochloromethane has been identified in air samples collected at 1 of the 174 NPL hazardous waste sites where it was detected in some environmental media.

The average concentration of dibromochloromethane in uncontrolled emissions from 40 medical waste incinerators in the United States and Canada was 0.96 μg/kg waste (Walker and Cooper 1992). The average concentration in controlled emissions was 536 μg/kg waste.

Quack and Wallace (2003) estimated that the annual global flux of bromoform of 3–22 Gmol/year with the main source being sea-to-air emissions from macroalgal and planktonic bromoform production. Estimated anthropogenic emission rates for bromoform are 34 Mmol/year from coastal power plants, 4 Mmol/year from inland nuclear power plants, 280 Mmol/year from coastal fossil fuel plants, 14 Mmol/year from inland fossil fuel plants, 2 Mmol/year from desalination power plants, 12 Mmol/year from water disinfection processes giving a total of 346 Mmol/year from all anthropogenic sources (Quack and Wallace 2003). Emissions of bromoform from a commercial rice paddy in Houston, Texas were reported to range from 0.012 to 0.032 $\mu g/m^2$/hour with a median emission rate of 0.021 $\mu g/m^2$/hour (Redeker et al. 2003). The mean annual concentrations of bromoform in air extracted from deep firn air

Table 6-1. Releases to the Environment from Facilities that Produce, Process, or Use Bromoform[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
AR	1	7	0	0	0	0	7	0	7
LA	1	0	456	0	0	0	456	0	456
NE	1	5	0	0	0	0	5	0	5
Total	3	12	456	0	0	0	468	0	468

Source: TRI02 2004 (Data are from 2002)

[a]The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list. Data are rounded to nearest whole number.
[b]Data in TRI are maximum amounts released by each facility.
[c]Post office state abbreviations are used.
[d]Number of reporting facilities.
[e]The sum of fugitive and point source releases are included in releases to air by a given facility.
[f]Surface water discharges, waste water treatment-(metals only), and publicly owned treatment works (POTWs) (metal and metal compounds).
[g]Class I wells, Class II-V wells, and underground injection.
[h]Resource Conservation and Recovery Act (RCRA) subtitle C landfills; other on-site landfills, land treatment, surface impoundments, other land disposal, other landfills.
[i]Storage only, solidification/stabilization (metals only), other off-site management, transfers to waste broker for disposal, unknown
[j]The sum of all releases of the chemical to air, land, water, and underground injection wells.
[k]Total amount of chemical transferred off-site, including to POTWs.

RF = reporting facilities; UI = underground injection

were 1.35 and 0.20 ppt at two Antarctic locations (Sturges et al. 2001). Mean annual concentrations of dibromochloromethane were 0.20 and 0.14 ppt.

No other studies were located regarding the amount of bromoform and dibromochloromethane released into the atmosphere from laboratories, chemical plants, or chemical waste sites. However, since neither compound is produced or used in large quantities (Perwak et al. 1980), atmospheric emissions from these sources are probably small.

6.2.2 Water

Estimated releases of 456 pounds (0.21 metric tons) of bromoform to surface water and publicly owned treatment works from three domestic manufacturing and processing facilities in 2002, accounted for about 97% of the estimated total environmental releases (TRI02 2004). These releases are summarized in Table 6-1.

Bromoform has been identified in surface water samples and groundwater samples collected at 14 and 103 of the 140 hazardous waste sites, respectively, where it was detected in some environmental media. Dibromochloromethane has been identified in surface water samples and groundwater samples collected at 15 and 146 of the 174 hazardous waste sites, respectively, where it was detected in some environmental media (HazDat 2005).

The principal anthropogenic source of bromoform and dibromochloromethane in the environment is chlorination of water containing organic materials (Bellar et al. 1974; EPA 1980a; Peters et al. 1994; Rook 1977; Rodriguez et al. 2004; Symons et al. 1975, 1993). It has been estimated that the total amounts of bromoform and dibromochloromethane generated by chlorinating U.S. drinking water in 1978 were 17 and 204 kkg, respectively (Perwak et al. 1980). Bromoform and dibromochloromethane were detected in the distribution systems of three water treatment plants that each use different methods of water chlorination (LeBel et al. 1997). Mean monthly concentrations of dibromochloromethane were 0.2 μg/L in samples from the chlorine-chloramine treatment plant, 0.3 μg/L in samples from the chlorine-chlorine treatment plant, and 0.1 μg/L in samples from the ozone-chlorine treatment plant. Mean monthly bromoform concentrations were <0.1 μg/L in samples from all three plants.

Unlike the chlorination of low-bromide fresh water where chloroform is the trihalomethane produced in the largest amounts, bromoform is the trihalomethane usually produced in the largest amounts during the

chlorination of sea water or high-bromide fresh water (Khalanski 2003; Richardson et al. 2003). The concentrations of bromoform in the chlorinated cooling water at three European marine power stations on the English Channel were 25.16, 11.4, and 18.23 μg/L (Khalanski 2003). Richardson et al. (2003) measured bromoform concentrations of 23.3, 57, 59, 44, and 55 μg/L in high-bromide water from the Sea of Galilee in Israel after a pre-chlorination step during the months of December, May, September, November, and July, respectively. Concentrations of dibromochloromethanein these samples were reported only for the months of May, September, and July (9.39, 1.9, and 3.7 μg/L, respectively).

Ozonation of water containing bromide ion has also been shown to be a source of bromoform in the environment (Glaze et al. 1989; Huang et al. 2003). Bromoform levels were 1.4, 3.7, and 2.1 mg/L in California State Project Water samples after ozonation at the Los Angeles Aqueduct Filtration Plant in 1987 at ozone doses of 1.0, 2.0, and 4.0 mg/L, respectively (Glaze et al. 1989). Huang et al. (2004) reported that bromoform generation is greater in waters with higher organic matter content.

Release of dibromochloromethane and bromoform into groundwater has been shown to occur as a consequence of the yearly aquifer storage and recovery (ASR) in the Las Vegas Valley. As an example, it is estimated that up to 16.3×10^6 m^3 water was injected into the underlying aquifer during the winter months of 1995–1996, when demand for water by neighboring Las Vegas was low (Thomas et al. 2000). Because the recharge water is chlorinated before injection, byproducts of this disinfection process are introduced into the groundwater at concentrations measured during the 1995–1996 ASR recharge seasons of 18 μg/L for dibromochloromethane and 2.6 μg/L for bromoform in the recharge water. During the water recovery phase beginning in the spring of 1996, initial concentrations of dibromochloromethane and bromoform in water recovered from one recharge well (#14) in the spring of 1996 were below detection limits (0.5 μg/L) but were much higher, 15 and 2.4 μg/L, respectively, in a second well (#51). Dibromochloromethane and bromoform levels in water recovered from the second well fell during the remaining spring and summer months decreasing to concentrations of 1.5 and 0.6 μg/L, respectively, in September of 1996. The differences in the concentrations of dibromochloromethane and bromoform between wells 14 and 51, and the decrease in concentrations observed in water recovered from well 51 during the spring and summer recovery months of 1996, were attributed to both dilution of the recharge water in the aquifer with natural water from the surrounding aquifer basin and biotransformation of the trihalomethanes within the aquifer.

Dibromochloromethane and bromoform may also occur as a consequence of chlorinating industrial waste waters (Perry et al. 1979). Staples et al. (1985) reported that bromoform was detected in 60 of

1,346 samples of industrial waste effluent, at a median concentration of <5 μg/L, and dibromochloromethane was detected in 84 of 1,298 samples at a median concentration of <2.4 μg/L. These values are not significantly higher than those for typical chlorinated water (see Section 5.4.2), suggesting that industrial discharge may not be a major source of release.

Bromoform and dibromochloromethane were detected in New York City municipal waste water samples taken from 1989 to 1993 (Stubin et al. 1996). Influent concentrations ranged from 7 to 40 μg/L for bromoform with a frequency of detection of 4% and 9 μg/L for dibromochloromethane with a frequency of detection of 1%. Effluent concentrations ranged from 3 to 11 μg/L for bromoform with a frequency of detection of 11% and from 2 to 15 μg/L for dibromochloromethane with a frequency of detection of 8%. Moschandreas et al. (1997) studied samples from 14 New York City waste water pollution control plants to determine the organic compound concentrations in the waste water at each of the plants. Bromoform was detected with concentrations of 0.4–10 μg/L in raw influent samples, 2.0–9.0 μg/L in primary influent samples, 1.0–10 μg/L in primary effluent samples, 0.3–11 μg/L in aeration tank samples, 0.4–12 μg/L in superficial of return activated sludge samples, and 0.2–12 μg/L in plant effluent samples. Only the plant effluent concentration range (0.3–5.0 μg/L) was reported for dibromochloromethane.

Although most raw water samples collected from different points of the Great Lakes in 1990 contained trihalomethanes at concentrations <2 μg/L, mean concentrations in treated water samples from four of the six locations were above 25 μg/L (Henshaw et al. 1993). The low trihalomethane concentrations of the other two locations are attributed to the use of alternative methods of water treatment such as ammonia addition and potassium permanganate disinfection.

The average concentration of dibromochloromethane in sewage treatment effluents in England and Wales in 1995 was 0.2007 μg/L with a frequency of detection of 30.4% (Stangroom et al. 1998a). The average concentration of dibromochloromethane in trade effluents in England and Wales in 1995 was 7.91 μg/L with a frequency of detection of 63%.

Mean dibromochloromethane concentrations were determined to be 2 ng/L in water and sediment samples taken from the anoxic fjord Idefjorden between Norway and Sweden near the city of Halden, Norway (Abrahamsson and Klick 1989). Dibromochloromethane was detected in effluent water from a nearby paper pulp mill.

Class et al. (1986) observed trace levels of dibromochloromethane and bromoform and other halogenated methanes in sea water (0.1–6 ng/L) and in the air (0.1–20 ppt) at several locations in the Atlantic. Other studies have reported atmospheric bromoform concentration ranges of 1.0–22.7 pptv off the coast of Galway, Ireland, 0.7–8.0 pptv off the coast of northwestern Tasmania, 0.28–2.9 pptv in the West Pacific Islands, 0.32–7.1 pptv in the Asian Seas, and 1.0–37.4 pptv off the Antarctic Peninsula (Carpenter et al. 1999, 2003). Dibromochloromethane was also detected off the coasts of Galway, Ireland and northwestern Tasmania with atmospheric concentrations of 0.13–1.8 and 0.1–1.4 pptv, respectively. The presence of these compounds can be attributed to biosynthesis and release of bromochloromethanes by macroalgae (Carpenter et al. 1999; Class et al. 1986; Gschwend et al. 1985; Marshall et al. 2003; Quack and Wallace 2003). Gschwend et al. (1985) estimated that marine algae could be a major global source of volatile organobromides. More recently, Carpenter and Liss (2000) estimated that macroalgae produce around 70% of the world's bromoform, which is greater than what was previously estimated due to updated seaweed biomass estimates. However, Class et al. (1986) states that this source accounts for <1% of the anthropogenic burden of total organohalogens in the atmosphere.

6.2.3 Soil

There were no releases to the ground either through soil or underground injection from three domestic manufacturing and processing facilities in 2002 (TRI02 2004). This information is summarized in Table 6-1.

Bromoform has been identified in soil and sediment samples collected at 26 and 3 of the 140 NPL hazardous waste sites, respectively, where it was detected in some environmental media. Dibromochloromethane has been identified in soil and sediment samples collected at 21 and 4 of the 174 NPL hazardous waste sites, respectively, where it was detected in some environmental media (HazDat 2005).

Soils and other unconsolidated surficial materials may become contaminated with bromoform and dibromochloromethane by chemical spills, the landfilling of halomethane-containing solid wastes, or the discharge of chlorinated water. However, no data were located to suggest that land releases are a significant source of the chemicals in the environment.

6.3 ENVIRONMENTAL FATE

6.3.1 Transport and Partitioning

Bromoform and dibromochloromethane are slightly volatile liquids, and tend to exist primarily as vapors in the atmosphere. The vapor pressure of bromoform is 0.007 atm at 20 °C (Mabey et al. 1982), and the vapor pressure of dibromochloromethane at 20 °C is approximately 0.1 atm (Mabey et al. 1982). The half-time of evaporation from flowing, aerated water (e.g., rivers and streams) has been estimated to range from 1 to 581 hours for bromoform and from 0.7 to 398 hours for dibromochloromethane (Kaczmar et al. 1984; Mackay et al. 1982).

Both dibromochloromethane and bromoform are moderately soluble in water (Callahan et al. 1979; Heikes 1987), and so each may be removed from the air by being dissolved into clouds or raindrops. Estimates of the Henry's law constant (H) (the tendency of a chemical to partition between its vapor phase and water) for bromoform range from 4.3 to 5.6×10^{-4} atm-m^3/mole, and from 8.7 to 9.9×10^{-4} atm-m^3/mole for dibromochloromethane (Mabey et al. 1982; Mackay and Shiu 1981; Munz and Roberts 1987; Nicholson et al. 1984). The magnitude of these values suggest that the two halomethanes will tend to partition to both water and air.

It is not known if either compound can be adsorbed by airborne particulate matter that is subject to atmospheric dispersion, gravitational settling, or wash-out by rain. Particle adsorption is probably not an important transport mechanism because these chemicals occur at such low concentrations in the atmosphere.

Bromoform and dibromochloromethane have a minor tendency to be adsorbed by soils and sediments. Calculated and measured values of K_{oc} (the organic carbon/water partition coefficient, an index of the relative mobility of a material in water-soil systems) for bromoform range from 62 to 126 (Hassett et al. 1983; Hutzler et al. 1986; Mabey et al. 1982). These relatively low values imply that bromoform will exhibit only a minor affinity for soil materials and will tend to be highly mobile (Roy and Griffin 1985). This low tendency for adsorption to soil has been confirmed in laboratory studies by Curtis et al. (1986) and in field studies by Roberts et al. (1986).

A similar K_{oc} value of 57 has been estimated for dibromochloromethane, based on its measured water solubility (Heikes 1987). No studies were located on the adsorption of dibromochloromethane by soils or

soil materials, but it is likely that dibromochloromethane will have properties generally similar to those of bromoform.

Bromoform and dibromochloromethane may be slightly bioconcentrated by aquatic organisms. The octanol/water partition coefficient (K_{ow}) (an index of the partitioning of a compound between octanol and water) is approximately 240 for bromoform and 170 for dibromochloromethane (Mabey et al. 1982). The magnitudes of these values suggest that the chemicals will tend to partition to fat tissues of aquatic organisms. No studies were located regarding the bioconcentration factor (BCF) for dibromochloromethane or bromoform, but based on measured BCFs for similar compounds (Kenaga 1980), the BCF of dibromochloromethane and bromoform may be on the order of 2–10. It is not known if these chemicals can be transferred through food chains to higher trophic levels, but this seems unlikely to be of major concern.

The percent removal of dibromochloromethane measured at the outlet of a waste-water-dependent constructed wetland near Phoenix, Arizona was 86.5% (Keefe et al. 2004). The authors concluded that volatilization was the primary removal mechanism.

6.3.2 Transformation and Degradation

6.3.2.1 Air

Based on the behavior of similar compounds, it seems likely that bromoform and dibromochloromethane may be degraded by photooxidative interactions with atmospheric OH radicals. Radding et al. (1977) proposed that the atmospheric half-life of bromoform and dibromochloromethane is approximately 1–2 months, but this has not been confirmed by direct experimental measurements.

6.3.2.2 Water

Both dibromochloromethane and bromoform are relatively stable in water, with estimated hydrolytic rate constants of 3.2×10^{-11} sec^{-1} and 8×10^{-11} sec^{-1} (Mabey and Mill 1978). These rate constants correspond to hydrolytic half-lives of 686 and 274 years for bromoform and dibromochloromethane, respectively.

No information was located on oxidation or photolysis of these chemicals in water, but it is not expected that either is a significant degradative pathway.

It has been found that dibromochloromethane and bromoform undergo only limited biodegradation (10–25%) under aerobic conditions, although the rate may increase somewhat after microbial adaptation (Bouwer et al. 1981; Tabak et al. 1981a). Bromoform, at initial concentrations ranging from 132 to 177 μg/L, underwent >99% reduction in a continuous-flow biofilm column seeded with primary settled sewage with 1.5 hours packed-bed detention time (Cobb and Bouwer 1991). Under anaerobic conditions, dibromochloromethane and bromoform have been found to be readily biodegraded in the presence of methane-producing bacteria (Bouwer and McCarty 1983a; Bouwer et al. 1981), and under denitrifying and sulfate-reducing conditions in batch and column experiments (Bouwer and McCarty 1983b; Bouwer and Wright 1986). There is also some field evidence that trihalomethanes degrade in anaerobic groundwater (Bouwer et al. 1981), with half-lives estimated to be between 21 and 42 days (Bouwer and McCarty 1984). Increased degradation rates were observed for bromoform and dibromochloromethane under conditions similar to those found inside ferrogenic aquifers, especially at higher pH (Kenneke et al. 2003; Pecher et al. 2002). Bouwer and Wright (1986) reported that one degradation product of bromoform was dibromomethane, but there was no additional information on the identity or fate of environmental degradation by-products. Bromoform was not degraded in enriched sea water cultures taken from beds of the giant kelp, *M. pyrifera* off the coast of California after 40 days (Goodwin et al. 1997).

The concentration of bromoform decreased from 58 μg/L to <1 μg/L in water stored in an aquifer 7 days after recharge with chlorinated water (Nicholson et al. 2002). The concentration of dibromochloromethane decreased from 46 to 3 μg/L 28 days after recharge. The authors stated that the main attenuation processes were most likely adsorption and degradation under methanogenic conditions. McQuarrie and Carlson (2003) suggest that aquifer storage may be a potential method for the removal of disinfection byproducts such as bromoform and dibromochloromethane from treated water.

6.3.2.3 Sediment and Soil

No studies were located regarding the biodegradation of dibromochloromethane or bromoform in soil. It is expected that observations regarding biodegradation rates in aerobic and anaerobic aqueous media (above) will be generally applicable to degradation rates in moist soils.

6.3.2.4 Other Media

No information was located on the transformation and degradation of bromoform or dibromochloromethane in other media.

6.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

Reliable evaluation of the potential for human exposure to bromoform and dibromochloromethane depends in part on the reliability of supporting analytical data from environmental samples and biological specimens. Concentrations of bromoform and dibromochloromethane in unpolluted atmospheres and in pristine surface waters are often so low as to be near the limits of current analytical methods. In reviewing data on bromoform and dibromochloromethane levels monitored or estimated in the environment, it should also be noted that the amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable. The analytical methods available for monitoring bromoform and dibromochloromethane in a variety of environmental media are detailed in Chapter 7.

6.4.1 Air

Dibromochloromethane and bromoform are usually found in air only at very low concentrations. Brodinsky and Singh (1983) tabulated data on dibromochloromethane and bromoform levels in ambient air from five urban locations across the United States. For dibromochloromethane, 63 of 89 samples were below the detection limit, the mean value was 3.8 ppt (32 ng/m^3), and the highest value was 27 ppt (230 ng/m^3). For bromoform, 60 of 78 samples were below the detection limit, the mean value was 3.6 ppt (37 ng/m^3) and the highest value was 71 ppt (730 ng/m^3). Forty-six air samples collected near four chemical plants in Arkansas contained a mean bromoform concentration of 0.9 ppt (9 ng/m^3) (Pellizzari 1978). The mean dibromochloromethane concentration was 0.08 ppt (0.8 ng/m^3), but 54 of 56 measurements were <0.05 ppt (0.5 ng/m^3).

Fischer et al. (2000) found a nearly constant mixing ratio of bromoform at 0.5 parts per trillion by volume (pptv) in the marine troposphere of the Atlantic across 30 °W longitude. In the coastal region of South America, bromoform concentrations were 2.4 pptv. A high concentration of bromoform (2.0 pptv) was found in an air sample taken near Cape Verde Island. Air mixing ratios for bromoform in samples taken above the waters of the equatorial Pacific during the third joint Soviet-American Gases and Aerosols

(SAGA 3) experiment ranged from 0.68 to 3.28 pptv using the charcoal or Tenax adsorption method and from 0.5 to 6.7 pptv using the canister collection method (Atlas et al. 1993). Air mixing ratios for dibromochloromethane ranged from 0.09 to 0.49 pptv using the charcoal or Tenax adsorption method. Dibromochloromethane was not measured with the canister collection method. The mean concentration of bromoform in ambient air samples collected in the Arctic Circle was 5.1 ppt (53 ng/m^3) (Berg et al. 1984).

During the Urban Air Toxics Monitoring Program, concentrations of bromoform and dibromochloromethane at 13 air monitoring sites located in Vermont, New Jersey, Louisiana, and Texas were <1 parts per billion by volume (ppbv) (no detection limit given) (Mohamed et al. 2002). Bromoform was not detected in air samples from three permanent sites in Arizona (Tucson, Payson, and Casa Grande) during the Arizona Hazardous Air Pollutants Monitoring Program, though the detection limit was not specified (Zielinska et al. 1998). Bromoform was detected at a fourth permanent site in Phoenix with a maximum concentration of 0.02 ppbv, although the average bromoform concentration at this site was below the detection limit. Bromoform was not detected (detection limit unspecified) in air samples collected from 13 semi-rural to urban locations in Maine, Massachusetts, New Jersey, Pennsylvania, Ohio, Illinois, Louisiana, and California (Pankow et al. 2003). However, dibromochloromethane was detected at Kettering, Ohio at a concentration of 0.007 ppbV. This concentration was below the lowest daily standard of 0.057 ppbV. Neither bromoform nor dibromochloromethane were detected (detection limit not specified) in air samples from 13 locations in Texas, Louisiana, New Jersey, and Vermont (Mohamed et al. 2002).

The mean, minimum, and maximum concentrations of dibromochloromethane in air samples taken during the Southern California Ozone Study in 1997 were 0.02 ppbv (210 ng/m^3), 0.04 ppbv (420 ng/m^3), and 0.01 ppbv (105 ng/m^3), respectively at a residential/industrial site in Mexicali, Mexico (Zielinska et al. 2001). Dibromochloromethane was not detected at a residential site in Rosarito, Mexico (no detection limit given). Dibromochloromethane was detected at 24 ppb (0.210 mg/m^3) in Finnish industrial air samples medical industry preparing solutions and at 1.6 ppb (0.014 μg/m^3) in suburban air from Turku, Finland (Kroneld 1989a, 1989b). Dibromochloromethane was not detected in countryside air samples taken from the Islands of Inio at the southwest coast of Finland. Mean concentrations of bromoform detected in air samples collected at two locations in the city of Kaohsiung, Taiwan during the spring of 2002 were 0.25 and 1.00 μg/m^3 (Lai et al. 2004).

Bromoform was detected with an occurrence rate of 54% in indoor air samples taken from 26 houses, although actual concentrations were not given (Kostiainen 1995). Air from 13 houses in Katusushika Ward, Tokyo, Japan were sampled during a survey of indoor pollution by volatile organohalogen compounds in February 1995 (Amagai et al. 1999). Thirty houses were sampled in July 1995. Bromoform was detected in all 13 houses during the February survey. In July, it was detected in 22 out of 30 houses. Dibromochloromethane was detected in all houses during both the February and July surveys. Mean concentrations of dibromochloromethane in February 1995 were 0.016 μg/m^3 in outdoor air, 0.095 μg/m^3 in living room air, 0.122 μg/m^3 in kitchen air, 0.096 μg/m^3 in bedroom air, and 0.701 μg/m^3 in bathroom air. Mean concentrations of dibromochloromethane in July 1995 were 0.007 ug/m^3 in outdoor air, 0.050 μg/m^3 in living room air, 0.065 μg/m^3 in kitchen air, 0.042 μg/m^3 in bedroom air, and 0.372 μg/m^3 in bathroom air. Mean concentrations of bromoform in February 1995 were 0.009 ug/m^3 in outdoor air, 0.016 μg/m^3 in living room air, 0.018 μg/m^3 in kitchen air, 0.015 μg/m^3 in bedroom air, and 0.091 μg/m^3 in bathroom air. Mean concentrations of bromoform in July 1995 were 0.010 μg/m^3 in outdoor air, 0.013 μg/m^3 in living room air, 0.013 μg/m^3 in kitchen air, 0.012 μg/m^3 in bedroom air, and 0.034 μg/m^3 in bathroom air.

Kerger et al. (2000) evaluated airborne concentrations of common trihalomethane compounds in bathrooms during showering and bathing in three urban homes supplied with chlorinated tap water. Samples were collected prior to, during, and after the water-use event for 16 shower and 7 bath events. The increases in average airborne concentration of dibromochloromethane were 0.5 μg/m^3 per μg/L during showers and 0.15 μg/m^3 per μg/L during baths.

No studies were located regarding atmospheric concentrations of bromoform or dibromochloromethane in the workplace. Dibromochloromethane was detected in air samples at two hazardous waste sites, but the amounts were not quantified (LaRegina et al. 1986).

6.4.2 Water

Dibromochloromethane and bromoform are rarely measurable in nonchlorinated water (Cech et al. 1981; Staples et al. 1985; Varma et al. 1984), but both are very frequently found in chlorinated water. The levels of bromoform and dibromochloromethane in finished (chlorinated) drinking water have been investigated in several studies (see Table 6-2). Except for a few cases, the concentrations of bromoform and dibromochloromethane in drinking water were <100 μg/L, with mean concentrations generally <10 μg/L.

Table 6-2. Occurrence of Bromoform and Dibromochloromethane in Finished Drinking Water

Frequency of detection percent	Concentration (µg/L) Range	Mean	Location	Reference
Bromoform				
10	ND–92	≈0.4	National	Symons et al. 1975
27	ND–3.0	≈0.5	13 Cities	Keith et al. 1976
34	NR	12	National	Brass et al. 1977
67	NR–4.4	0.4	Midwest	ORVWSC 1979
NR	ND–258	≈7	Texas	Glaze and Rawley 1979
100	4–17	9	Texas	Smith et al. 1980
26	NR–110	NR	National	Westrick et al. 1984
NR	1–10	NR	Iowa	Kelley et al. 1985
8	ND–1.6	0.1	Michigan	Furlong and D'Itri 1986
NR	NR	0.8–8	California	Wallace et al. 1986b
Dibromochloromethane				
86	<0.1–2	0.9	Ohio	Bellar et al. 1974
NR	3-32	NR	5 Cities	Coleman et al. 1975
37	ND–110	2.7	National	Symons et al. 1975
85	ND–15.0	≈0.4	13 Cities	Keith et al. 1976
65	ND–9.0	2.9	Iowa	Morris and Johnson 1976
86	NR	14	National	Brass et al. 1977
99	NR–33	5.6	Midwest	ORVWSC 1979
NR	ND–128	≈20	Texas	Glaze and Rawley 1979
100	11–31	20	Texas	Smith et al. 1980
42	NR–63	NR	National	Westrick et al. 1984
NR	1–28	NR	Iowa	Kelley et al. 1985
75	ND–40	4.1	Michigan	Furlong and D'Itri 1986
NR	NR	1–2	New Jersey	Wallace et al. 1986a
NR	NR	8–28	California	Wallace et al. 1986b

µg = microgram; L = liter; ND = not detected; NR = not reported

Bromoform and dibromochloromethane were detected in approximately 90% of samples from 5,320 wells in 21 states from 1985 to 1995 according to data collected by the United States Geological Survey (USGS) National Water-Quality Assessment Program, although actual concentrations were not given (Lapham et al. 2000). Bromoform and dibromochloromethane were detected in 27 and 39 out of 7,712 California drinking water wells, respectively, during monitoring studies conducted from 1984 to 1990 (Lam et al. 1994). Maximum concentrations were 78 μg/L for bromoform and 30 μg/L for dibromochloromethane. Dibromochloromethane was detected in 5 out of 6 samples from 178 active public supply wells in the Los Angeles physiographic basin, California above the laboratory reporting limit of 0.18 μg/L in August through September 2000 (Shelton et al. 2001). Bromoform was detected in four out of four samples above the laboratory reporting limit of 0.06 μg/L. Clark et al. (1994) collected drinking water samples from different points along the North Marin Water District, which serves the greater Novato area in California. Bromoform and dibromochloromethane were detected in the samples with concentrations ranging from 2.4 to 11.4 μg/L and from <0.1 to 7.5 μg/L, respectively. The concentrations of bromoform and dibromochloromethane were below their detection limits of 0.06 and 0.18 μg/L, respectively, in samples collected from 30 groundwater monitoring wells in Wichita, Kansas (USGS 2002). The wells were located in areas of recent residential and commercial development.

The State of California's Water Quality Monitoring Database contains data for public drinking water systems that use either groundwater or surface water sources (Storm 1994). Analysis of these data shows that bromoform was detected in 145 out of 11,765 samples (1.2%) and dibromochloromethane was detected in 171 out of 11,782 samples (1.5%). Mean concentrations in samples with detected dibromochloromethane and bromoform were 8.95 and 7.63 μg/L, respectively. Occurrence data from approximately 22,000 public water systems (source water: 88% groundwater and 12% surface water) from a cross-section of 24 states were selected from the Unregulated Contaminant Information System database (Round 1 monitoring data) (EPA 2001a). Dibromochloromethane and bromoform were detected in these systems with 99th percentile values of 12.7 and 7.3 μg/L, respectively. Occurrence data from approximately 27,000 public water systems (source water: 89% groundwater and 11% surface water) from a cross-section of 22 states were selected from the Safe Drinking Water Information System database (Round 2 monitoring data). Dibromochloromethane and bromoform were detected in these systems with 99th percentile values of 9.7 and 6.5 μg/L, respectively.

It is usually found that halomethanes occur at higher concentrations in drinking water derived from surface sources than those from groundwater supplies because the former tends to contain more dissolved

organic matter (Bellar et al. 1974; Cech et al. 1981; Glaze and Rawley 1979; Page 1981). The total trihalomethane content of finished water from a given facility can be extremely variable as a function of time (Arguello et al. 1979; Smith et al. 1980), with lower levels of halomethanes usually occurring during the winter.

The mean concentration of dibromochloromethane was 0.74 μg/L in cold season samples from the Elizabethtown, New Jersey water distribution system (Chen and Weisel 1998). Dibromochloromethane levels rose to 1.7 μg/L in warm season samples. Bromoform was not found above the detection limit (0.10 μg/L) in cold season samples from the distribution system, but it was detected in warm season samples with a mean concentration of 0.27 μg/L. Summer concentration ranges in treated water immediately prior to distribution in a 1993 national Canadian survey conducted by Health Canada were <0.1–19.8 μg/L for dibromochloromethane and <0.1–4.2 μg/L for bromoform (Ritter et al. 2002). Winter concentration ranges were <0.1–9.0 μg/L for dibromochloromethane and <0.1–3.3 μg/L for bromoform. Bromoform was detected in the influent, treated, and distribution stages from 47 municipal water treatment plants in Ontario, Canada at concentrations of 0.5, 0–7.0, and 0.2–8.5 μg/L, respectively.

Dibromochloromethane was detected in samples from plant effluents of 35 Finnish waterworks with concentrations ranging from <0.05 to 3 μg/L (Nissinen et al. 2002). One exception was a comparatively high concentration of 43 μg/L measured at one of the plants. Bromoform concentrations were <0.05 μg/L in samples from all plants except for three (0.48, 0.31, and 27 μg/L). Concentrations of bromoform and dibromochloromethane in samples from two Finnish waterworks rose in the spring, changed little over summer and fall, and then dropped during winter. Campillo et al. (2004) detected bromoform in tap water samples collected in Spanish cities at concentrations ranging from 1.8 to 24.7 μg/L (mean=13.5 μg/L). The concentrations of dibromochloromethane in these samples ranged from 2.0 to 66.5 μg/L (mean=30 μg/L). Mean concentrations of dibromochloromethane and bromoform were 0.5 μg/L and below the detection limit (0.3 μg/L), respectively, in tap water from the city of Cherepovets, Russia (Egorov et al. 2003). Dibromochloromethane was detected in tap water samples collected from the 19 districts of Hong Kong at concentrations ranging from 0.830 to 4.15 μg/L (Lee et al. 2004b). Bromoform was detected (detection limit 0.03 μg/L) in tap water samples from only 4 of the 19 districts at concentrations ranging from 0.040 to 0.920 μg/L.

Bromoform and dibromochloromethane were detected in water samples from seven out of nine household residences in the Lower Rio Grande Valley during the spring of 1993 with concentrations ranging from 1.0 to 14.1 μg/L and from 3.3 to 17.3 μg/L, respectively (Berry et al. 1997). These compounds were

detected in water samples from five out of nine residences in the same region during the summer of 1993 with concentrations ranging from 1.6 to 31.7 μg/L and from 1.8 to 49.9 μg/L, respectively.

In order to reduce trihalomethane and related disinfection byproduct concentrations in treated water, communities have begun employing treatment methods that remove disinfection byproduct precursors such as natural organic carbon from the water prior to chlorination (Dey et al. 2001; Peltier et al. 2002; Shetty and Chellam 2003; Weiss et al. 2003; Westerhoff et al. 2004). Some processes employ a coagulation step to aid in filtration or adsorption (Dey et al. 2001; Drikas et al. 2003). Disinfection byproduct removal was studied in the two trains of the Mery-sur-Oise treatment plant, which feeds the northern Parisian Suburbs (Peltier et al. 2002). The newer train uses nanofiltration technology while the older train employs conventional treatment. Trihalomethane concentrations in water treated by the nanofiltration train were 50% less than trihalomethane concentrations in water treated by the conventional train. Weiss et al. (2002, 2003) observed that levels of total organic carbon and dissolved organic carbon in water from the Ohio, Wabash, and Missouri Rivers were reduced by 35–67% after riverbank filtration. However, the brominated trihalomethane precursors were not removed as efficiently as the chlorinated trihalomethane precursors (<40% compared to 80%). This was attributed to the increase in the bromide to total organic carbon ratio as the water traveled from the rivers to the collection wells.

Trihalomethanes may also form in chlorinated swimming pools (Beech et al. 1980). For fresh water pools, chloroform and dichlorobromomethane were usually the predominant THM species present, with dibromochloromethane and bromoform averaging 3–15 and 1–2 μg/L, respectively. However, in saline pools (which have a higher bromide ion content than fresh water pools), bromoform was the major THM present (average concentration of 650 μ/L), with lower concentrations (5–27 μg/L) of dibromochloromethane, bromodichloromethane, and chloroform. Concentrations of bromoform and dibromochloromethane were 1.8–2.9 and 3.5–17 μg/L, respectively, in samples taken from three chlorinated sandy bottom swimming areas in September (Mansour et al. 1999). The concentrations of dibromochloromethane were below 40 μg/L in water samples from eight swimming pools in Nagpur, India (Thacker and Nitnaware 2003). Bromoform was detected in samples from only one of the pools at a concentration below 10 μg/L.

Dibromochloromethane and bromoform have also been detected in water near hazardous waste sites, although this is not common. Data from the Contract Laboratory Program (CLP) Statistical Data Base (CLPSD 1988) indicated that bromoform was detected in surface water at 2 of 862 hazardous-waste sites; the median concentration was 7 μg/L. Dibromochloromethane was detected in only one sample

(45 µg/L). Bromoform was detected in groundwater samples collected at four sites; the median concentration was 26 µg/L. Based on monitoring data from 479 disposal site groundwater investigations that were conducted across the United States, bromoform was detected in groundwater samples from 10 hazardous waste disposal sites and dibromochloromethane has been detected in groundwater samples from 8 hazardous waste disposal sites (Plumb 1991). Actual concentrations of bromoform and dibromochloromethane were not given.

In a random survey of 954 community water systems, bromoform and dibromochloromethane were detected (minimum reporting limit of 0.2 µg/L) in 3.4 and 5.5%, respectively, of source water samples (Delzer and Ivahnenko 2003). Both bromoform and dibromochloromethane were detected more frequently in groundwater sources than surface water sources. Concentrations of bromoform in groundwater ranged from 0.19 to 2.61 µg/L with a median value of 0.26 µg/L; in surface water, the concentrations ranged from 0.03 to 0.06 µg/L with a median value of 0.05 µg/L. For dibromochloromethane, concentrations in groundwater ranged from 0.02 to 2.43 µg/L with a median value of 0.04 µg/L; in surface water, the concentrations ranged from 0.02 to 0.15 µg/L with a median value of 0.03 µg/L. In a study of over 21 cities throughout the United States conducted between 1991–1995, dibromochloromethane was detected (minimum reporting limit of 0.2 µg/L) in 3.4% of storm water and 0.5% of shallow groundwater samples (Lopes and Bender 1998). The presence of dibromochloromethane in these samples was attributed to the irrigation of lawns, gardens, and parks with chlorinated water, the draining of swimming pools, and uses of chlorinated water that result in its introduction to storm water catchments.

Dibromochloromethane has been detected at concentrations ranging from 1.1 to 36.3 ng/L in the Scheldt River estuary, Germany during May 1998; from 0.2 to 22.7 ng/L in Scheldt River estuary, Germany during October 1998; from below the detection limit to 39.0 ng/L in the Thames River estuary, England during February 1999; from below the detection limit to 7.7 ng/L in the Loire River estuary, France during September 1998; and from below the detection limit to 9.8 ng/L in the Rhine River estuary, Germany during November 1997 (Christof et al. 2002). Bromoform and dibromochloromethane were detected in water samples from the Southampton Water estuary at concentrations of 10–2,597 and 10–2,200 ng/L, respectively (Bianchi et al. 1991).

Bromoform was not found above a detection limit of 0.08 µ/L in 644 samples from 46 surface water locations, including sea, estuarine, river, and industrial effluent, throughout Portugal (Martinez et al. 2002). Concentrations of bromoform in the Belaya River, Russia were <0.0001 mg/L above an industrial zone and <0.0004 mg/L below the industrial zone (Safarova et al. 2004). The concentration of

bromoform in the waste water flowing into this river was measured as 0.0021 mg/L. The concentrations of bromoform and dibromochloromethane in finished water from the city of Hanoi, Vietnam ranged from 1.2 to 8.5 μg/L (average, 6.6 μg/L) and from 0.3 to 22.3 μg/L (average, 6.3 μg/L), respectively, in high bromide finished water and from <0.2 to 3.7 μg/L (average, 0.5 μg/L) and from <0.2 to 3.8 μg/L (average, 1.8 μg/L), respectively, in low bromide finished water (Duong et al. 2003). In high bromide finished water that also had high ammonia content, dibromochloromethane was detected at concentrations ranging from <0.2 to 3.7 μg/L (average, 0.3 μg/L), while the concentration of bromoform was <0.2 μg/L. Bromoform and dibromochloromethane have been detected in the Marta River, Italy at concentrations of 2.39–3.35 and 0.04–0.11 μg/L, respectively (Russo et al. 2003). The concentrations of bromoform and dibromochloromethane in the Tiber River, Italy were 0.82–1.82 and <16–0.11 μg/L, respectively.

6.4.3 Sediment and Soil

Staples et al. (1985) reported that bromoform was not detected in any of 353 sediment samples analyzed. No data were available for dibromochloromethane. Data from the Contract Laboratory Program Statistic Data Base (CLPSD 1988) indicated dibromochloromethane and bromoform were detected in soils in only 2 of 862 hazardous waste sites; the median concentrations were 17 μg/kg (bromoform) and 15 μg/kg (dibromochloromethane). Bromoform and dibromochloromethane were detected in sediment samples from the Southampton Water estuary at concentrations ranging from 75 to 62,609 and from 150 to 27,350 ng/kg, respectively (Bianchi et al. 1991). During a monitoring study of volatile halogenated organic compounds in the Klosterhede State Forest District in Jutland, Denmark, bromoform was the only brominated compound detected in soil air samples and only in the upper soil layer with a concentration of 0.25 ng/L (Laturnus et al. 2000b).

6.4.4 Other Environmental Media

The use of chlorinated water in the manufacture of commercially bottled drinks has raised concern over the presence of trihalomethanes in these drinks (McNeal et al. 1995). Levels of dibromochloromethane in bottled water and in carbonated soft drinks ranged from 0.5 to 2 ng/g in a survey of soft drinks, juices, beers, bottled water, and canned foods purchased from Washington, DC markets in 1991–1992. Bromoform was not found in any of the samples above a detection limit of 0.1 ng/g. Campillo et al. (2004) detected bromoform in pineapple juice, apple juice, and forest fruits juice at concentrations of 4.2,

3.5, and 2.5 μg/L, respectively. Dibromochloromethane was found in pasteurized milk from a suburban area of Turku, Finland (Kroneld and Reunanen 1990).

Neither bromoform nor dibromochloromethane were detected in oyster, clam, or sediment samples from the Ariho and Yoshingaga Rivers in Japan (Gotoh et al. 1992). No detection limit was given. The mean concentrations of bromoform and dibromochloromethane in daily dietary samples collected from housewives in Nogoya and Yokohama, Japan were 0.2–0.6 and <0.1–0.2 ng/g, respectively (Toyoda et al. 1990).

6.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

Because of the variability of dibromochloromethane and bromoform concentrations in water and air, it is not possible to derive precise estimates of typical human exposure levels. However, based on the typical ranges of dibromochloromethane and bromoform concentrations measured in water and air, it is likely that most individuals will be exposed to average doses of <1 μg/kg/day (Table 6-3), nearly all of which is from water. Limited data suggest that exposure levels around chemical factories or waste sites are not likely to be much higher, but this can only be evaluated on a site-by-site basis.

The range of dibromochloromethane concentrations in tap water, in blood samples before showering, and in blood samples after showering in Cobb County, Georgia were <1–4, 0.001–0.003, and 0.003–0.029 μg/L, respectively (Miles et al. 2002). Concentrations in tap water, in blood samples before showering, and in blood samples after showering samples from Corpus Christi, Texas were 5–20, 0.002–0.031, and 0.011–>0.093 μg/L, respectively. Bromoform was detected in the Cobb County, Georgia samples at concentrations of <1 μg/L in tap water, 0.001–0.0052 μg/L in blood before showering, and 0.0001–0.0059 μg/L in blood after showering. Concentrations of dibromochloromethane in tap water, in blood samples before showering, and in blood samples after showering from Corpus Christi, Texas ranged from 2–17, 0.001–0.02, and 0.006–0.064 μg/L, respectively. Breath samples of individuals taken within 5 minutes after a shower with high dibromochloromethane and bromoform water concentrations contained dibromochloromethane at a mean concentration of 4.8 $\mu g/m^3$ and bromoform at a mean concentration of 2.3 $\mu g/m^3$ (Weisel 1999). Breath samples taken 5–20 minutes after a shower with high water concentration contained dibromochloromethane at a mean concentration of 2.8 $\mu g/m^3$ and bromoform at a mean concentration of 1.2 $\mu g/m^3$. Breath samples taken >20 minutes after a shower with high water concentration contained dibromochloromethane at a mean concentration of 1 $\mu g/m^3$ and bromoform at a mean concentration of 0.6 $\mu g/m^3$. Breath samples from individuals after a shower with

Table 6-3. Summary of Typical Human Exposure to Bromoform and Dibromochloromethane

Parameter	Exposure medium	
	Water	Air
Typical concentration in medium	1–20 μg/L	0–0.1 μg/m^3
Assumed intake of medium by 70-kg adult	2 L/day	20 m^3/day
Assumed absorption fraction	1.0	0.5
Estimated dose to 70-kg adult	0.03–0.6 μg/kg/day	0.0–0.01 μg/kg/day

μg = microgram; kg = kilogram; L = liter; m^3 = cubic meters

low water concentration contained dibromochloromethane with a mean concentration of 1 μg/m^3 at different collection times and bromoform with a mean concentration of 0.6 μg/m^3 at different collection times.

Blood samples taken from 16 individuals in the Lower Rio Grande Valley during the spring of 1993 had a maximum dibromochloromethane concentration of 0.05 μg/L (38% frequency of detection) and a maximum bromoform concentration of 0.07 μg/L (19% frequency of detection) (Buckley et al. 1997). Blood samples taken during the Third National Health and Nutrition Examination Survey, which included over 1,000 individuals, had a maximum dibromochloromethane concentration of 0.024 μg/L (14.5% frequency of detection) and a maximum bromoform concentration of 0.034 μg/L (9.9% frequency of detection) (Buckley et al. 1997; Needham et al. 1995).

Dibromochloromethane was found in human kidney and lung tissue at 0.6 and 0.06 μg/kg, respectively, in samples taken in the city of Turku, Finland in 1987 (Kroneld 1989a). It was also detected in air samples from that region at a concentration of 0.31 mg/m^3. Polkowska et al. (2003) studied the relationship between concentrations of volatile organic halogens in drinking water and the concentrations of these substances in the urine of individuals living in Gdansk-Sopot-Gdynia Tri City area of Poland. Concentrations of dibromochloromethane in drinking water from three locations were 0.015–0.159, 0.912–1.125, and 0.953–0.987 μg/L with corresponding urine concentrations ranging from not detected to 0.003 μg/L, from 0.001 to 0.012 μg/L, and from not detected to 0.005 μg/L, respectively. Bromoform was only detected in drinking water from two locations at concentrations of 0.018–0.032 and 0.018–0.045 μg/L. It was not detected in any of the urine samples. Detection limits were not provided in this study.

The mean concentrations of bromoform and dibromochloromethane in daily dietary samples collected from housewives in Nogoya and Yokohama, Japan were 0.2–0.6 and <1–0.2 ng/g, respectively (Toyoda et al. 1990). Based on these concentrations, the mean daily dietary intakes for housewives in these cities were calculated to be 0.5–1.2 μg for bromoform and 0–0.5 μg for dibromochloromethane.

Exposure to dibromochloromethane and bromoform may be above average for persons who swim in chlorinated swimming pools. Beech (1980) estimated that the total dose for a 6-year-old boy who swam for 3 hours in a pool containing 500 μg/L of trihalomethanes could be as high as 2.8 mg (130 μg/kg). About 60% of this dose was attributed to dermal absorption, with about 30% resulting from inhalation. In fresh water pools, only a small fraction of this would be dibromochloromethane or bromoform, but in a

salt water pool, a large fraction would be expected to be bromoform (Beech et al. 1980). Aggazzotti et al. (1998) studied the exposure of competitive indoor swimmers to trihalomethanes. The mean concentrations of dibromochloromethane were 0.8 μg/L in the pool water, 5.2 μg/m^3 in the pool air prior to swimming, and 11.4 μg/m^3 in pool air during swimming. The mean concentration of bromoform was 0.1 μg/L in the pool water. Bromoform was detected above 0.1 μg/m^3 in only one air sample. Mean concentrations of dibromochloromethane in the alveolar air sampled from five swimmers were 0.8 μg/m^3 before swimming and 1.4 μg/m^3 after 1 hour of swimming. Bromoform was not quantified in any of the alveolar air samples. Calculated dibromochloromethane intakes for the five swimmers were 1.5–1.9 μg/hour before swimming and 14–22 μg/hour after 1 hour of swimming.

Individuals who work at indoor pool facilities are expected to be at risk for occupational exposure to trihalomethanes (Fantuzzi et al. 2001). Dibromochloromethane was detected in 24 out of 32 alveolar air samples collected from individuals who work at selected indoor swimming pools in Modena, Italy (Fantuzzi et al. 2001). The mean concentration was 0.5 μg/m^3. Bromoform was not detected in any of the alveolar air samples above the detection limit of 0.1 μg/m^3. The authors reported that the concentration of trihalomethanes in the alveolar air of poolside attendants was double that in alveolar air collected from employees working in the engine room and reception area (25.1 vs. 14.8 μg/m^3). Mean concentrations of dibromochloromethane measured in this study were 1.9 μg/L in pool water, 3.1 μg/m^3 in poolside ambient air, 1.5 μg/m^3 in reception area ambient air, and 1.6 μg/m^3 in engine room ambient air. The mean concentration of bromoform was 0.4 μg/L in pool water. It was only detected in one ambient air sample (poolside) at a concentration of 0.8 μg/m^3.

6.6 EXPOSURES OF CHILDREN

This section focuses on exposures from conception to maturity at 18 years in humans. Differences from adults in susceptibility to hazardous substances are discussed in Section 3.7, Children's Susceptibility.

Children are not small adults. A child's exposure may differ from an adult's exposure in many ways. Children drink more fluids, eat more food, breathe more air per kilogram of body weight, and have a larger skin surface in proportion to their body volume. A child's diet often differs from that of adults. The developing human's source of nutrition changes with age: from placental nourishment to breast milk or formula to the diet of older children who eat more of certain types of foods than adults. A child's behavior and lifestyle also influence exposure. Children crawl on the floor, put things in their mouths,

sometimes eat inappropriate things (such as dirt or paint chips), and spend more time outdoors. Children also are closer to the ground, and they do not use the judgment of adults to avoid hazards (NRC 1993).

No information was located regarding the exposures of children to bromoform or dibromochloromethane. Exposures of children to bromoform and dibromochloromethane through inhalation are expected to vary depending on the length of time a child spends indoors and the concentrations of these compounds in indoor air. The concentrations of bromoform and dibromochloromethane will depend on the source of household water, exchange rate of indoor and outdoor air, frequency and duration of showers, and emission rates of these compounds from other household sources (e.g., dishwasher, washing machines, etc.). Dermal exposures of children to bromoform and dibromochloromethane are expected to be lower than, or similar to, those found for adults, depending on frequency and duration of bathing or showering. However, the primary routes of exposure to bromoform and dibromochloromethane are expected to be through drinking water or consumption of beverage or food products that contain water that has been disinfected through chlorination. It is expected that a child's exposure to bromoform and dibromochloromethane will depend predominantly on the source and amount of drinking water consumed per day. Exposure of newborns and infants to bromoform and dibromochloromethane, whose diets are supplemented with human breast milk, cow's milk, or infant formulas, is not known, since measurements of these compounds are not available in these media. Swimming is expected to provide an additional source of exposure for children who spend time in chlorinated swimming pools (Beech et al. 1980).

6.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

The environmental medium most likely to be contaminated with bromoform and dibromochloromethane is chlorinated water. Therefore, any person who is in frequent contact with such water could have above average exposures. This includes individuals who drink large quantities of water, such as workers in hot climates, or individuals with swimming pools or saunas, where contact could occur by inhalation or by dermal contact. Since bromoform and dibromochloromethane levels in water depend on the organic content of the source water before chlorination, individuals whose water source is high in organics are likely to have finished water with higher-than-average bromoform and dibromochloromethane levels.

Workers in chemical production facilities or laboratories where bromoform and dibromochloromethane is made or used would also have potentially high exposures to the chemicals, most likely by inhalation or dermal exposure. Persons living near hazardous-waste sites may have potentially high exposures to bromoform and dibromochloromethane, but this can only be evaluated on a case-by-case basis.

6.8 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of bromoform and dibromochloromethane is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of bromoform and dibromochloromethane.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.8.1 Identification of Data Needs

Physical and Chemical Properties. The physical and chemical properties of dibromochloromethane and bromoform have been well studied, and reliable values for key parameters are available for use in environmental fate and transport models. On this basis, it does not appear that further studies of the physical-chemical properties of dibromochloromethane and bromoform are essential.

Production, Import/Export, Use, Release, and Disposal. According to the Emergency Planning and Community Right-to-Know Act of 1986, 42 U.S.C. Section 11023, industries are required to submit substance release and off-site transfer information to the EPA. The TRI, which contains this information for 2002, became available in May of 2004. This database is updated yearly and should provide a list of industrial production facilities and emissions.

Available data indicate that neither bromoform nor dibromochloromethane is produced or used in significant quantities in the United States. Nevertheless, a listing of laboratories or industries that use small amounts in research or testing would be valuable in identifying locations where the potential for

environmental releases or human exposure exists. Also, information on the means of disposal of waste chemicals would be valuable in identifying environmental media likely to be affected at such sites. Federal regulations do restrict disposal of dibromochloromethane and bromoform to land or in industrial effluents.

Environmental Fate. The fate of dibromochloromethane and bromoform in the environment has not been thoroughly studied, although the physical-chemical properties indicate that both are likely to partition to air and water. Volatilization rates have been calculated for flowing rivers and streams, but direct measurements of half-times of volatilization would be useful, both for surface waters and for household water (showers, baths, cooking, etc.). Adsorption of these compounds to soils and sediments has been studied and does not appear to be a significant factor. Consequently, transport in surface or groundwater are likely to be important. Studies to confirm these expectations and provide more precise descriptions of the environmental behavior of these compounds would be valuable in assessing human exposure near specific sources of release.

Degradation of dibromochloromethane and bromoform in air has not been studied, but is expected to occur by reaction with hydroxyl radicals. Studies to measure the atmospheric half-times of these compounds would be valuable in estimating long-term trends in atmospheric levels, but such studies are probably not essential in estimating exposure near specific sources. Neither chemical undergoes chemical degradation in water, but both are subject to microbial breakdown in water (especially anaerobic groundwater) or moist soils. Further data on the rate of microbial degradation of dibromochloromethane and bromoform in water and soil would be valuable, with special attention to how these rates depend on environmental conditions (oxygen level, pH, etc.).

Bioavailability from Environmental Media. Both dibromochloromethane and bromoform are known to be absorbed following oral and inhalation exposure. No data are available regarding dermal absorption, but it seems likely that uptake across the skin may occur. No data were located regarding the relative bioavailability of dibromochloromethane and bromoform in water, soil or air. Because of their physical and chemical properties, it is expected that the bioavailability of dibromochloromethane and bromoform are not significantly reduced by environmental media, but studies to substantiate this presumption would be helpful.

Food Chain Bioaccumulation. There are few data on bioconcentration of dibromochloromethane or bromoform by plants or aquatic organisms, and no data were located on the bioaccumulation of

bromoform and dibromochloromethane in the food chain. This lack of data may not be a major limitation because the general levels of the chemicals in water and soil appear to be quite low, and based on the K_{oc} of these chemicals, there appears to be a low likelihood of food chain buildup.

Exposure Levels in Environmental Media. Reliable monitoring data for the levels of bromoform and dibromochloromethane in contaminated media at hazardous waste sites are needed so that the information obtained on levels of bromoform and dibromochloromethane in the environment can be used in combination with the known body burden of bromoform and dibromochloromethane to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

There are several studies on the atmospheric concentrations of bromoform and dibromochloromethane in urban and rural environments, but many of the samples did not have detectable levels. No data on levels in air near waste sites were located. More research in this area using more sensitive analytical methods would be helpful, although it is anticipated that typical atmospheric levels will usually be low enough that air is not the principal route of exposure. Data are available on dibromochloromethane and bromoform in a number of chlorinated drinking water systems, and these compounds have been detected in surface water and groundwater near a few hazardous waste sites. Further studies on the levels of these compounds in water and soil around waste sites would be valuable in evaluating the risk to human health posed by these contaminants.

Exposure Levels in Humans. There are no data on levels of dibromochloromethane or bromoform in blood, breath or other tissues from humans residing near waste sites. Low levels of bromoform have been detected in blood of humans, presumably as the result of exposure through ingestion of chlorinated drinking water. Levels in expired breath and in adipose tissue appear to be too low to measure reliably for the general population. Direct measurement of typical human intake from water and air (especially indoor air) would be helpful in obtaining more accurate estimates of typical human dose levels. Similar data on inhalation and dermal doses would be useful for bromoform and dibromochloromethane in and around swimming pools (especially indoor pools).

This information is necessary for assessing the need to conduct health studies on these populations.

Exposures of Children. No formal studies of children's inhalation, dermal, or oral exposures to bromoform and dibromochloromethane were located in the literature. Based on the concentrations of bromoform and dibromochloromethane measured in indoor air and in drinking water that has been

disinfected by chlorination, studies are needed to assess the inhalation, dermal, and oral exposures of children to these and other disinfection by-products. Data on inhalation and dermal doses would be useful for bromoform and dibromochloromethane in and around swimming pools (especially indoor pools).

Child health data needs relating to susceptibility are discussed in Section 3.12.2, Identification of Data Needs: Children's Susceptibility.

Exposure Registries. No exposure registries for bromoform and dibromochloromethane were located. These compounds are not currently one of the compounds for which a sub-registry has been established in the National Exposure Registry. These compounds will be considered in the future when chemical selection is made for sub-registries to be established. The information that is amassed in the National Exposure Registry facilitates the epidemiological research needed to assess adverse health outcomes that may be related to exposure to this substance.

6.8.2 Ongoing Studies

The Federal Research in Progress (FEDRIP 2004) database provides additional information obtainable from a few ongoing studies that may fill in some of the data needs identified in Section 6.8.1. These studies are summarized in Table 6-4.

Table 6-4. Ongoing Studies on the Potential for Human Exposure to Bromoform[a]

Investigator	Affiliation	Research description	Sponsor
Harrington, J; Simpson WR	Pennsylvania State University, University Park, Pennsylvania; University of Alaska Fairbanks Campus, Fairbanks, Alaska	Development and testing of a radiation-transfer model for snow photochemistry. This will help quantify the extent of photochemical processing of trace compounds within snow and will help in interpreting ice core climate records and understanding atmospheric chemistry in snow-covered regions. The study will include measurements of photolysis rates for bromoform.	National Science Foundation
Meyer GJ	Johns Hopkins University, Baltimore, Maryland	Development of new reductive and oxidative dehalogenation chemistries and the elucidation of their fundamental mechanisms; application of these new findings to the sensing, remediation, and determination of the environmental fate of organohalide pollutants. The study will provide a pedagogical platform that informs and educates the next generation of environmental chemists.	National Science Foundation
Saltzman E	University of California-Irvine	The degradation of natural halogenated compounds in coastal seawater will be studied. Measurements will be used to revise estimates for the uptake of atmospheric halocarbons by the oceans, the extent to which the oceans can buffer atmospheric halocarbon conc entrations, and the production rate for these gases in the surface oceans.	National Science Foundation

[a]Source: CRIS 2003; FEDRIP 2003, 2004

7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring bromoform and dibromochloromethane, its metabolites, and other biomarkers of exposure and effect to bromoform and dibromochloromethane. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision.

As is true for most volatile organic compounds, the preferred analytical technique for dibromochloromethane and bromoform is gas chromatography (GC) (Ashley et al. 1996; Djozan and Assadi 1995; Fishbein 1985). A number of devices are suitable for detection and quantification of dibromochloromethane and bromoform as they emerge from the GC, including flame ionization detection (GC/FID), halogen-sensitive detection (GC/HSD) or electron-capture detection (GC/ECD). In general, HSD or ECD are preferable because of their high sensitivity for halogenated compounds. When absolute confidence in compound identity is required, mass spectrometry (GC/MS) is the method of choice.

The most variable aspect of analyses of this sort is the sample preparation procedure used to separate dibromochloromethane and bromoform from the test medium in order to prepare a sample suitable for GC analysis. As volatile organic compounds of relatively low water solubility, both dibromochloromethane and bromoform are easily lost from biological and environmental samples, so appropriate care must be exercised in handling and storing such samples for chemical analysis. Brief summaries of the methods available for extraction and detection of these compounds in biological and environmental samples are provided below.

7.1 BIOLOGICAL MATERIALS

Separation of dibromochloromethane and bromoform from biological samples is most often achieved by headspace analysis, purge-and-trap collection, solvent extraction, or direct collection on adsorbent resins.

Headspace analysis offers speed, simplicity, and good reproducibility, but partitioning of the analyte between the headspace and the sample matrix is dependent upon the nature of the matrix and must be determined separately for each different kind of matrix (Walters 1986).

Purge-and-trap collection is well suited to biological samples such as blood or urine that are readily soluble in water (Ashley et al. 1996; Peoples et al. 1979). This method consists of bubbling an inert gas through a small volume of the sample and collecting the vapor in a trap packed with sorbent. The analytes are then removed from the trap by heating it and backflushing the analytes onto a gas chromatographic column. The two materials most widely used for adsorption and thermal desorption of volatile organic compounds collected by the purge and trap technique are Carbotrap®, consisting of graphitized carbon black, and Tenax®, a porous polymer of 2,6-diphenyl-p-phenylene oxide (Fabbri et al. 1987).

For water-insoluble materials such as fat or other tissues, the most common separation procedure is extraction with an organic solvent such as diethyl ether (Zlatkis and Kim 1976). Homogenization of tissue with the extractant and lysing of cells usually improves solvent extraction efficiency.

Analytical methods for the determination of bromoform and dibromochloromethane in biological materials are summarized in Table 7-1.

7.2 ENVIRONMENTAL SAMPLES

Dibromochloromethane and bromoform may be isolated from environmental samples using the same methods and principles as those used for biological materials, followed by gas chromatographic analysis. The most convenient procedure for most liquid and solid samples is the purge-and-trap method. Arthur et al. (1992) used solid phase micro extraction to separate volatile halogenated compounds from water samples. The organic analytes partition between the water sample and the stationary phase coating of a fused silica fiber before they are thermally desorbed in a GC injector. Djozan and Assadi (1995) introduced a gas stripping cryogenic trapping technique for separating volatile halogenated compounds from drinking water samples. In this method, a purified gas passes through the water sample in a stripping column where it removes volatile compounds from a water sample. The effluent from the column is dried, trapped in a cold trapping coil, and then released to the GC system by warming. Membrane inlet mass spectrometry (MIMS) is another technique for separating organohalogen compounds from drinking water (Bocchini et al. 1999). With this method, volatile organic compounds

Table 7-1. Analytical Methods for Determining Bromoform and Dibromochloromethane in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Accuracy (percent recovery)	Reference
Adipose tissue	Extraction, bulk lipid removal, Florisil fractionation	HRGC/MS	0.1 µg/g	NR	Mack and Stanley 1985
Adipose tissue	Heated dynamic headspace purge-and-trap	HRGC/MS	1 ng/g (DBCM) 2 ng/g (TBM)	NR	Stanley 1986
Adipose tissue	Purge from liquified fat at 115 °C, trap on Tenax/silica gel, thermal desorption	GC/HSD	<2 µg/L	83–107 (TBM) 90–118 (DBCM)	Peoples et al. 1979
Blood	Purge from blood onto Tenax, thermal desorption onto column maintained at -20 °C	GC/MS	≈0.1 ng/mL	NR	Antoine et al. 1986
Blood	Extract with n-pentane	HRGC/ECD	0.1 µg/L (DBCM)	NR	Kroneld 1985
Blood	Purge from blood at 30 °C, trap onto Tenax, thermal desorption	GC/MS	0.021µg/L (TBM)	102–108 (TBM)	Ashley et al. 1992
Blood	Purge from blood at 30 °C, trap onto Tenax, thermal desorption	GC/MS	0.017µg/L (DBCM)	91–104 (DBCM)	Ashley et al. 1992
Blood, tissue	Macerate tissue in water, warm blood or tissue, pass inert gas through, trap on Tenax, thermal desorption	GC/MS	3 ng/mL (blood) 6 ng/g (tissue)	NR	Pellizzari et al. 1985b
Blood serum	Purge from water-serum mixture containing antifoam reagent at 115 °C, trap on Tenax/silica gel, thermal desorption	GS/HSD	<2 µg/L	79–100 (TBM) 78–100 (DBCM)	Peoples et al. 1979
Breath	Trap on Tenax, dry over calcium sulfate, thermal desorption	GC/MS	1–5 µg/m^3	92±15 (TBM) 93±13 (DBCM)	Wallace et al. 1986b

µg = microgram; DBCM = dibromochloromethane; ECD = electron capture detector; g = gram; GC = gas chromatography; HRGC = high resolution gas chromatography; HSD = halide specific detector; L = liter; m^3 = cubic meters; mg = milligram; mL = milliliter; MS = mass spectrometry; ng = nanogram; NR = not reported; TBM = bromoform

from the drinking water sample diffuse through a hollow-fiber membrane into a mass spectrometer. The advantages of this method are that sample pre-treatment is not required, response times are fast, and trace analysis of the pollutants can be preformed on-line. Halocarbons can also be removed from water by adsorption on synthetic polymers contained in cartridges, followed by thermal desorption of the analyte (Pankow et al. 1988). Among the products used for this purpose are Tenax-GC® and Tenax-TA®. A similar procedure is used for air, in which the air is passed through an adsorbent canister, followed by thermal desorption (Pankow et al. 1998).

Analytical methods for the determination of dibromochloromethane and bromoform in environmental samples are given in Table 7-2.

7.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of bromoform and dibromochloromethane is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of bromoform and dibromochloromethane.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect.

Exposure. Sensitive and specific methods exist for the determination of dibromochloromethane and bromoform in blood, expired air, and adipose tissue. These methods are presumably sensitive enough to

Table 7-2. Analytical Methods for Determining Bromoform and Dibromochloromethane in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Accuracy (percent recovery)	Reference
Drinking water	Solvent extraction with pentane, direct injection of extract	HRGC/ECD	<0.5 µg/L	NR	Fayad and Iqbal 1985
Drinking water	Gas stripping and cryogenic trapping system	GC/FID	0.05 µg/L (DBCM)	75±7.7 (DBCM)	Djozan and Assadi 1995
Drinking water	Adsorption to and diffusion through a polymeric hollow-fiber membrane	MIMS	0.5 µg/L (TBM)	NR	Bocchini et al. 1999
Drinking water	Adsorption to and diffusion through a polymeric hollow-fiber membrane	MIMS	0.5 µg/L (DBCM)	NR	Bocchini et al. 1999
Drinking water	Purge and trap, thermal desorption	GC/MS	0.1µg/L	89–90 (TBM)	Eichelberger et al. 1990
Drinking water	Purge and trap, thermal desorption	GC/MS	0.1µg/L	95–100 (TBM)	Eichelberger et al. 1990
Air	Coconut shell charcoal sorption, carbon disulfide desorption	GC/FID	10 µg/sample (TBM)	14.0 (TBM)	NIOSH 1994
Air	Carbotrap/Carboxen filled glass cartridge adsorption/thermal desorption	GC/MS	0.02 ppbv (TBM)	95–102 (TBM)	Pankow et al. 1998
Air	Carbotrap/Carboxen filled glass cartridge adsorption/thermal desorption	GC/MS	0.04 ppbv (DBCM)	96–99 (DBCM)	Pankow et al. 1998
Water	Purge and trap	GC/MS	10 µg/L	NR	EPA 1980b
Water	Purge and trap	GC/HSD	0.20 µg/L (TBM)	89±9 (TBM)	EPA 1982a
			0.09 µg/L (DBCM)	98±7 (DBCM)	
Water	Purge and trap	GC/MS	4.7 µg/L (TBM)	105±16 (TBM)	EPA 1982b
			3.1 µg/L (DBCM)	104±14 (DBCM)	
Water	Purge and trap	GC/HSD	0.5 µg/L	97 (DBCM)	APHA 1985a
				101 (DBCM)	
Water	Purge and trap	GC/MS	<2 µg/L	82 (TBM)	APHA 1985b
Water	Solvent extraction (isooctane)	GC/ECD	2 µg/L	NR	ASTM 1988
Water	Solid phase micro extraction	GC/MS	4.7 µg/L (TBM)		Arthur et al. 1992

Table 7-2. Analytical Methods for Determining Bromoform and Dibromochloromethane in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Accuracy (percent recovery)	Reference
Water	Solid phase micro extraction	GC/MS	3.1 µg/L (DBCM)		Arthur et al. 1992
Contaminated soil	Purge and trap	GC/HSD	2 µg/kg (TBM)	96[b] (TBM)	EPA 1986a
			0.9 µg/kg (DBCM)	94[b] (DBCM)	
Wastes, nonwater miscible	Purge and trap	GC/HSD	250 µg/kg (TBM)	96[b] (TBM)	EPA 1986a
			113 µg/kg (DBCM)	94[b] (DBCM)	
Solid waste	Purge and trap	GC/MS	5 µg/kg	118[b] (TBM)	EPA 1986b
				101[b] (DBCM)	

[a]Value refers to both DBCM and TBM unless noted otherwise.
[b]This recovery is typical at concentrations of around 100 µg/L or higher. Recoveries may deviate at lower concentrations.

µg = microgram; DBCM = dibromochloromethane; ECD = electron capture detector; FID = flame ionization detector; g = gram; GC = gas chromatography; HRGC = high resolution gas chromatography; HSD = halide specific detector; kg = kilogram; L = liter; MS = mass spectrometry; NR = not reported; TBM = bromoform

measure levels in humans exposed to doses of the chemicals that produce sedation or cause injury to liveand kidney. However, data on this are lacking due to absence of cases. The methods are also suitable for measuring background levels in the general population, although increased sensitivity would be useful for analysis of expired air and adipose tissue. The major limitation to these methods is that only recent exposures can be detected, so work to identify and quantify a more stable biomarker of exposure (e.g., a halomethyl adduct) would be valuable.

Effect. No chemical or biochemical biomarkers of effect are recognized, aside from nonspecific indices of hepatic or renal dysfunction, Efforts to identify a specific biomarker of effect (in particular, an effect such as alkylation of DNA that may be related to cancer risk) would be valuable in evaluating potential health risk to exposed humans.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Reliable and specific methods exist for measuring parent dibromochloromethane and bromoform in air, water, soil and solid wastes. Humans could be exposed to these compounds by contact with any of these media, although ingestion of or dermal contact with contaminated water appears to be the most likely route near a chemical waste site. Existing methods are readily able to detect concentration values in environmental media that are likely to lead to significant noncancer health effects, but might not be sensitive enough to measure levels that pose low levels of cancer risks. However, since no chemical-specific cancer potency values are available for these components, this is not certain.

7.3.2 Ongoing Studies

No ongoing studies on analytical methods were identified in a search of the Federal Research in Progress database (FEDRIP 2004).

8. REGULATIONS AND ADVISORIES

The international, national, and state regulations and guidelines regarding bromoform and dibromochloromethane in air, water, and other media are summarized in Table 8-1.

An acute-duration oral MRL of 0.7 mg/kg/day has been derived for bromoform. This MRL is based on a NOAEL of 72 mg/kg/day and a LOAEL of 145 mg/kg/day for centrilobular pallor in mice receiving gavage doses of bromoform for 14 days (Condie et al. 1983). The MRL was derived by dividing the NOAEL by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability).

An intermediate-duration oral MRL of 0.2 mg/kg/day has been derived for bromoform. This MRL is based on a NOAEL of 50 mg/kg for hepatocellular vacuolization in rats administered gavage doses of bromoform in corn oil 5 days/week for 13 weeks (NTP 1989a). The MRL was derived by dividing the duration adjusted NOAEL of 18 mg/kg/day by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability).

A chronic-duration oral MRL of 0.02 mg/kg/day has been derived for bromoform. This MRL is based on a LOAEL of 100 mg/kg for hepatocellular vacuolization in rats administered gavage doses of bromoform in corn oil 5 days/week for 2 years (NTP 1989a). The MRL was derived by dividing the duration adjusted LOAEL of 71 mg/kg/day by an uncertainty factor of 300 (3 for use of a minimal LOAEL, 10 for animal to human extrapolation, and 10 for human variability) and a modifying factor of 10 to account for the identification of a lower LOAEL in a 13-week study (NTP 1989a).

An acute-duration oral MRL of 0.1 mg/kg/day has been derived for dibromochloromethane. This MRL is based on a LOAEL of 37 mg/kg for hepatocellular vacuolization in mice administered gavage doses of dibromochloromethane in corn oil for 14 days (Condie et al. 1983). The MRL was derived by dividing the LOAEL by an uncertainty factor of 300 (3 for use of a minimal LOAEL, 10 for animal to human extrapolation, and 10 for human variability).

A chronic-duration oral MRL of 0.09 mg/kg/day has been derived for dibromochloromethane. This MRL is based on a LOAEL of 40 mg/kg for fatty changes in the liver of rats administered gavage doses of dibromochloromethane in corn oil for 2 years (NTP 1985). The MRL was derived by dividing the

duration-adjusted LOAEL of 28 mg/kg/day by an uncertainty factor of 300 (3 for use of a minimal LOAEL, 10 for animal to human extrapolation, and 10 for human variability).

Table 8-1. Regulations and Guidelines Applicable to Bromoform and Dibromochloromethane

Agency	Description	Information	Reference
INTERNATIONAL Guidelines:			
IARC	Carcinogenicity classification		IARC 1999a, 1999b
	Bromoform	Group 3[a]	
	Dibromochloromethane	Group 3[a]	
WHO	Drinking water guideline		WHO 1996
	Bromoform	100 mg/L	
	Dibromochloromethane	100 mg/L	
NATIONAL Regulations and Guidelines:			
a. Air:			
ACGIH	TLV (8-hour TWA)		ACGIH 2003
	Bromoform[b]	0.5 ppm	
NIOSH	REL (10-hour TWA)		NIOSH 2003
	Bromoform[c]	0.5 ppm	
	IDLH	850 ppm	
OSHA	PEL (8-hour TWA) for general industry		OSHA 2003a 29 CFR 1910.1000, Table Z-1
	Bromoform[d]	0.5 ppm	
	PEL (8-hour TWA) for construction industry		OSHA 2003c 29 CFR 1926.55, Appendix A
	Bromoform[d]	0.5 ppm	
	PEL (8-hour TWA) for shipyard industry		OSHA 2003b 29 CFR 1915.1000
	Bromoform[d]	0.5 ppm	
USC	Hazardous air pollutant	Bromoform	USC 2003
b. Water			
EPA	Drinking water health advisories		EPA 2002a, 2002b
	Bromoform		
	1-day (10-kg child)	5.0 mg/L	
	10-day (10-kg child)	2.0 mg/L	
	DWEL[e]	0.7 mg/L	
	10^{-4} Cancer risk[f]	0.4 mg/L	
	Dibromochloromethane		
	1-day (10-kg child)	6.0 mg/L	
	10-day (10-kg child)	6.0 mg/L	
	DWEL[e]	0.7 mg/L	
	Lifetime[g]	0.06 mg/L	
	10^{-4} Cancer risk[f]	0.04 mg/L	
	Effluent guidelines and standards; toxic pollutants pursuant to Section 307 (a)(1) of the Clean Water Act	Bromoform	EPA 2003c 40 CFR 401.15

Table 8-1. Regulations and Guidelines Applicable to Bromoform and Dibromochloromethane

Agency	Description	Information		Reference
NATIONAL *(cont.)*				
EPA	National primary drinking water regulations			EPA 2003f 40 CFR 141.12
	MCL for total trihalomethanes[h]	0.10 mg/L		
	Pollutants of initial focus in the Great Lakes Water Quality Initiative	Bromoform and dibromochloromethane		EPA 2003m 40 CFR 132, Table 6
c. Food				
FDA	Bottled drinking water allowable concentrations for total trihalo-methanes[i]	0.10 mg/L		FDA 2003 21 CFR 165.110
d. Other				
ACGIH	Carcinogenicity classification	A3[j]		ACGIH 2003
EPA	Carcinogenicity classification			IRIS 2004a, 2004b
	Bromoform	B2[k]		
	Dibromochloromethane	C[l]		
	RfC	No data		IRIS 2004a, 2004b
	RfD			IRIS 2004a, 2004b
	Bromoform	2.0×10^{-2} mg/kg/day		
	Dibromochloromethane	2.0×10^{-2} mg/kg/day		
	Community right-to-know; release report; effective date of reporting			EPA 2003i 40 CFR 372.65
	Bromoform	01/01/87		
	Identification and listing of hazardous waste; hazardous waste number			EPA 2003d 40 CFR 261, Appendix VIII
	Bromoform	U225		
	Land disposal restrictions; universal treatment standards	Waste water (mg/L)	Non-waste water mg/kg)	EPA 2003e 40 CFR 268.48
	Bromoform	0.63	15	
	Dibromochloromethane	0.57	15	
	Municipal solid waste landfills; hazardous constituents	Suggested method	PQL (µg/L)	EPA 2003a 40 CFR 258, Appendix II
	Bromoform	8010	2	
		8021	15	
		8260	5	
	Dibromochloromethane	8010	1	
		8021	0.3	
		8260	5	
	Reportable quantity of hazardous substance in accordance with Section 307 (a) of the Clean Water Act, Section 112 of RCRA, and Section 112 of the Clean Air Act			EPA 2003b 40 CFR 302.4
	Bromoform	100 pounds		

Table 8-1. Regulations and Guidelines Applicable to Bromoform and Dibromochloromethane

Agency	Description	Information		Reference
NATIONAL *(cont.)*				
EPA	Reportable quantity of hazardous substance in accordance with Section 112 of RCRA			EPA 2003b 40 CFR 302.4
	Dibromochloromethane	100 pounds		
	Standards for the management of specific hazardous waste and types of hazardous waste management facilities			EPA 2003g 40 CFR 266, Appendix V
		Risk specific doses		
	Bromoform	7.0×10^{-1} µg/m^3		
	Standards for owners or operators of hazardous waste TSD facilities; maximum concentration for groundwater protection	Suggested method	PQL (µg/L)	EPA 2003h 40 CFR 264, Appendix IX
	Bromoform	8010	2	
		8240	5	
	Dibromochloromethane	8010	1	
		8240	5	
	TSCA; chemical information rules	Effective date	Sunset date	EPA 2003k 40 CFR 712.30
	Bromoform	03/11/94	05/10/94	
	TSCA; health and safety data reporting	Effective date	Sunset date	EPA 2003j 40 CFR 716.120
	Bromoform	06/01/87	06/01/97	
	Dibromochloromethane	06/01/87	06/01/87	
	TSCA; identification of specific chemical substance and mixture testing requirements for			EPA 2003l 40 CFR 799.5055
	Bromoform	Hydrolysis testing		
STATE				
a. Air	No data			
b. Water				
Arizona	Drinking water guideline			HSDB 2004a, 2004b
	Bromoform	0.19 µg/L		
	Dibromochloromethane	0.19 µg/L		
Florida	Drinking water guideline			HSDB 2004a, 2004b
	Bromoform	4.0 µg/L		
	Dibromochloromethane	1.0 µg/L		
Minnesota	Drinking water guideline			HSDB 2004a, 2004b
	Bromoform	40 µg/L		
	Dibromochloromethane	10 µg/L		
New Hampshire	Drinking water guideline			HSDB 2004a
	Bromoform	4.0 µg/L		
Wisconsin	Drinking water guideline			HSDB 2004a, 2004b
	Bromoform	4.4 µg/L		
	Dibromochloromethane	60 µg/L		

Table 8-1. Regulations and Guidelines Applicable to Bromoform and Dibromochloromethane

Agency	Description	Information	Reference
STATE *(cont.)*			
c. Food	No data		
d. Other	No data		

[a]Group 3: not classifiable as to its carcinogenicity to humans
[b]Skin notation: refers to the potential significant contribution to the overall exposure by the cutaneous route, including mucous membranes and the eyes, either by contact with vapors or, of probable greater significance, by direct skin contact with the substance.
[c]Skin designation: indicates the potential for dermal absorption; skin exposure should be prevented as necessary through the use of good work practices and gloves, coveralls, goggles, and other appropriate equipment.
[d]Skin designation
[e]DWEL: a lifetime exposure concentration protection of adverse, non-cancer health effects, that assumes all of the exposure to a contaminant is from drinking water.
[f]10^{-2} Cancer risk: the concentration of a chemical in drinking water corresponding to an excess estimated lifetime cancer risk of 1 in 10,000.
[g]Lifetime: the concentration of a chemical in drinking water that is not expected to cause any adverse noncarcinogenic effects for a lifetime of exposure. The Lifetime HA is based on exposure of a 70-kg adult consuming 2 L water/day.
[h]Total trihalomethanes (the sum of the concentrations of bromoform, dibromochloromethane, bromodichloromethane, and chloroform) applies to subpart H community water systems which serve a population of 10,000 people or more until December 31, 2001. This level applies to community water systems that use only groundwater not under the direct influence of surface water and serves a population of 10,000 people or more until December 31, 2003. Compliance with the MCL for total trihalomethanes is calculated pursuant to 40 CFR 141.30. After December 31, 2003, this section no longer applies.
[i]Total trihalomethanes: sum of the concentration in mg/L of the trihalomethane compounds (bromoform, dibromochloromethane, bromodichloromethane, and chloroform), rounded to two significant figures.
[j]A3: confirmed animal carcinogen with unknown relevance to humans
[k]B2: probable human carcinogen
[l]C: possible human carcinogen

ACGIH = American Conference of Governmental Industrial Hygienists; CFR = Code of Federal Regulations; DWEL = drinking water equivalent level; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; HSDB = Hazardous Substances Data Bank; IARC = International Agency for Research on Cancer; IDLH = immediately dangerous to life or health; IRIS = Integrated Risk Information System; MCL = maximum contaminant level; NIOSH = National Institute for Occupational Safety and Health; OSHA = Occupational Safety and Health Administration; PEL = permissible exposure limit; PQL = practical quantitation level; RCRA = Resource Conservation and Recovery Act; REL = recommended exposure limit; RfC = inhalation reference concentration; RfD = oral reference dose; TLV = threshold limit values; TSCA = Toxic Substances Control Act; TSD = treatment, storage, and disposal; TWA = time-weighted average; USC = United States Code; WHO = World Health Organization

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10. GLOSSARY

Absorption—The taking up of liquids by solids, or of gases by solids or liquids.

Acute Exposure—Exposure to a chemical for a duration of 14 days or less, as specified in the Toxicological Profiles.

Adsorption—The adhesion in an extremely thin layer of molecules (as of gases, solutes, or liquids) to the surfaces of solid bodies or liquids with which they are in contact.

Adsorption Coefficient (K_{oc})—The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

Adsorption Ratio (Kd)—The amount of a chemical adsorbed by sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

Benchmark Dose (BMD)—Usually defined as the lower confidence limit on the dose that produces a specified magnitude of changes in a specified adverse response. For example, a BMD_{10} would be the dose at the 95% lower confidence limit on a 10% response, and the benchmark response (BMR) would be 10%. The BMD is determined by modeling the dose response curve in the region of the dose response relationship where biologically observable data are feasible.

Benchmark Dose Model—A statistical dose-response model applied to either experimental toxicological or epidemiological data to calculate a BMD.

Bioconcentration Factor (BCF)—The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

Biomarkers—Broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility.

Cancer Effect Level (CEL)—The lowest dose of chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or tumors) between the exposed population and its appropriate control.

Carcinogen—A chemical capable of inducing cancer.

Case-Control Study—A type of epidemiological study that examines the relationship between a particular outcome (disease or condition) and a variety of potential causative agents (such as toxic chemicals). In a case-controlled study, a group of people with a specified and well-defined outcome is identified and compared to a similar group of people without outcome.

Case Report—Describes a single individual with a particular disease or exposure. These may suggest some potential topics for scientific research, but are not actual research studies.

Case Series—Describes the experience of a small number of individuals with the same disease or exposure. These may suggest potential topics for scientific research, but are not actual research studies.

Ceiling Value—A concentration of a substance that should not be exceeded, even instantaneously.

Chronic Exposure—Exposure to a chemical for 365 days or more, as specified in the Toxicological Profiles.

Cohort Study—A type of epidemiological study of a specific group or groups of people who have had a common insult (e.g., exposure to an agent suspected of causing disease or a common disease) and are followed forward from exposure to outcome. At least one exposed group is compared to one unexposed group.

Cross-sectional Study—A type of epidemiological study of a group or groups of people that examines the relationship between exposure and outcome to a chemical or to chemicals at one point in time.

Data Needs—Substance-specific informational needs that if met would reduce the uncertainties of human health assessment.

Developmental Toxicity—The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Dose-Response Relationship—The quantitative relationship between the amount of exposure to a toxicant and the incidence of the adverse effects.

Embryotoxicity and Fetotoxicity—Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the insult occurs. The terms, as used here, include malformations and variations, altered growth, and *in utero* death.

Environmental Protection Agency (EPA) Health Advisory—An estimate of acceptable drinking water levels for a chemical substance based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

Epidemiology—Refers to the investigation of factors that determine the frequency and distribution of disease or other health-related conditions within a defined human population during a specified period.

Genotoxicity—A specific adverse effect on the genome of living cells that, upon the duplication of affected cells, can be expressed as a mutagenic, clastogenic, or carcinogenic event because of specific alteration of the molecular structure of the genome.

Half-life—A measure of rate for the time required to eliminate one half of a quantity of a chemical from the body or environmental media.

Immediately Dangerous to Life or Health (IDLH)—The maximum environmental concentration of a contaminant from which one could escape within 30 minutes without any escape-impairing symptoms or irreversible health effects.

Immunologic Toxicity—The occurrence of adverse effects on the immune system that may result from exposure to environmental agents such as chemicals.

Immunological Effects—Functional changes in the immune response.

Incidence—The ratio of individuals in a population who develop a specified condition to the total number of individuals in that population who could have developed that condition in a specified time period.

Intermediate Exposure—Exposure to a chemical for a duration of 15–364 days, as specified in the Toxicological Profiles.

***In Vitro*—**Isolated from the living organism and artificially maintained, as in a test tube.

***In Vivo*—**Occurring within the living organism.

Lethal Concentration$_{(LO)}$ (LC_{LO})—The lowest concentration of a chemical in air that has been reported to have caused death in humans or animals.

Lethal Concentration$_{(50)}$ (LC_{50})—A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose$_{(LO)}$ (LD_{Lo})—The lowest dose of a chemical introduced by a route other than inhalation that has been reported to have caused death in humans or animals.

Lethal Dose$_{(50)}$ (LD_{50})—The dose of a chemical that has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time$_{(50)}$ (LT_{50})—A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL)—The lowest exposure level of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Lymphoreticular Effects—Represent morphological effects involving lymphatic tissues such as the lymph nodes, spleen, and thymus.

Malformations—Permanent structural changes that may adversely affect survival, development, or function.

Minimal Risk Level (MRL)—An estimate of daily human exposure to a hazardous substance that is likely to be without an appreciable risk of adverse noncancer health effects over a specified route and duration of exposure.

Modifying Factor (MF)—A value (greater than zero) that is applied to the derivation of a Minimal Risk Level (MRL) to reflect additional concerns about the database that are not covered by the uncertainty factors. The default value for a MF is 1.

Morbidity—State of being diseased; morbidity rate is the incidence or prevalence of disease in a specific population.

Mortality—Death; mortality rate is a measure of the number of deaths in a population during a specified interval of time.

Mutagen—A substance that causes mutations. A mutation is a change in the DNA sequence of a cell's DNA. Mutations can lead to birth defects, miscarriages, or cancer.

Necropsy—The gross examination of the organs and tissues of a dead body to determine the cause of death or pathological conditions.

Neurotoxicity—The occurrence of adverse effects on the nervous system following exposure to a chemical.

No-Observed-Adverse-Effect Level (NOAEL)—The dose of a chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Effects may be produced at this dose, but they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{ow})—The equilibrium ratio of the concentrations of a chemical in *n*-octanol and water, in dilute solution.

Odds Ratio (OR)—A means of measuring the association between an exposure (such as toxic substances and a disease or condition) that represents the best estimate of relative risk (risk as a ratio of the incidence among subjects exposed to a particular risk factor divided by the incidence among subjects who were not exposed to the risk factor). An OR of greater than 1 is considered to indicate greater risk of disease in the exposed group compared to the unexposed group.

Organophosphate or Organophosphorus Compound—A phosphorus-containing organic compound and especially a pesticide that acts by inhibiting cholinesterase.

Permissible Exposure Limit (PEL)—An Occupational Safety and Health Administration (OSHA) allowable exposure level in workplace air averaged over an 8-hour shift of a 40-hour workweek.

Pesticide—General classification of chemicals specifically developed and produced for use in the control of agricultural and public health pests.

Pharmacokinetics—The dynamic behavior of a material in the body, used to predict the fate (disposition) of an exogenous substance in an organism. Utilizing computational techniques, it provides the means of studying the absorption, distribution, metabolism, and excretion of chemicals by the body.

Pharmacokinetic Model—A set of equations that can be used to describe the time course of a parent chemical or metabolite in an animal system. There are two types of pharmacokinetic models: data-based and physiologically-based. A data-based model divides the animal system into a series of compartments, which, in general, do not represent real, identifiable anatomic regions of the body, whereas the physiologically-based model compartments represent real anatomic regions of the body.

Physiologically Based Pharmacodynamic (PBPD) Model—A type of physiologically based dose-response model that quantitatively describes the relationship between target tissue dose and toxic end points. These models advance the importance of physiologically based models in that they clearly describe the biological effect (response) produced by the system following exposure to an exogenous substance.

Physiologically Based Pharmacokinetic (PBPK) Model—Comprised of a series of compartments representing organs or tissue groups with realistic weights and blood flows. These models require a variety of physiological information: tissue volumes, blood flow rates to tissues, cardiac output, alveolar ventilation rates, and possibly membrane permeabilities. The models also utilize biochemical information, such as air/blood partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

Prevalence—The number of cases of a disease or condition in a population at one point in time.

Prospective Study—A type of cohort study in which the pertinent observations are made on events occurring after the start of the study. A group is followed over time.

q_1*—The upper-bound estimate of the low-dose slope of the dose-response curve as determined by the multistage procedure. The q_1* can be used to calculate an estimate of carcinogenic potency, the incremental excess cancer risk per unit of exposure (usually μg/L for water, mg/kg/day for food, and μg/m^3 for air).

Recommended Exposure Limit (REL)—A National Institute for Occupational Safety and Health (NIOSH) time-weighted average (TWA) concentration for up to a 10-hour workday during a 40-hour workweek.

Reference Concentration (RfC)—An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer health effects during a lifetime. The inhalation reference concentration is for continuous inhalation exposures and is appropriately expressed in units of mg/m^3 or ppm.

Reference Dose (RfD)—An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure of the human population to a potential hazard that is likely to be without risk of deleterious effects during a lifetime. The RfD is operationally derived from the no-observed-adverse-effect level (NOAEL, from animal and human studies) by a consistent application of uncertainty factors that reflect various types of data used to estimate RfDs and an additional modifying factor, which is based on a professional judgment of the entire database on the chemical. The RfDs are not applicable to nonthreshold effects such as cancer.

Reportable Quantity (RQ)—The quantity of a hazardous substance that is considered reportable under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Reportable quantities are (1) 1 pound or greater or (2) for selected substances, an amount established by regulation either under CERCLA or under Section 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

Reproductive Toxicity—The occurrence of adverse effects on the reproductive system that may result from exposure to a chemical. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

Retrospective Study—A type of cohort study based on a group of persons known to have been exposed at some time in the past. Data are collected from routinely recorded events, up to the time the study is undertaken. Retrospective studies are limited to causal factors that can be ascertained from existing records and/or examining survivors of the cohort.

Risk—The possibility or chance that some adverse effect will result from a given exposure to a chemical.

Risk Factor—An aspect of personal behavior or lifestyle, an environmental exposure, or an inborn or inherited characteristic that is associated with an increased occurrence of disease or other health-related event or condition.

Risk Ratio—The ratio of the risk among persons with specific risk factors compared to the risk among persons without risk factors. A risk ratio greater than 1 indicates greater risk of disease in the exposed group compared to the unexposed group.

Short-Term Exposure Limit (STEL)—The American Conference of Governmental Industrial Hygienists (ACGIH) maximum concentration to which workers can be exposed for up to 15 minutes continually. No more than four excursions are allowed per day, and there must be at least 60 minutes between exposure periods. The daily Threshold Limit Value-Time Weighted Average (TLV-TWA) may not be exceeded.

Standardized Mortality Ratio (SMR)—A ratio of the observed number of deaths and the expected number of deaths in a specific standard population.

Target Organ Toxicity—This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen—A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV)—An American Conference of Governmental Industrial Hygienists (ACGIH) concentration of a substance to which most workers can be exposed without adverse effect. The TLV may be expressed as a Time Weighted Average (TWA), as a Short-Term Exposure Limit (STEL), or as a ceiling limit (CL).

Time-Weighted Average (TWA)—An allowable exposure concentration averaged over a normal 8-hour workday or 40-hour workweek.

Toxic Dose$_{(50)}$ (TD$_{50}$)—A calculated dose of a chemical, introduced by a route other than inhalation, which is expected to cause a specific toxic effect in 50% of a defined experimental animal population.

Toxicokinetic—The absorption, distribution, and elimination of toxic compounds in the living organism.

Uncertainty Factor (UF)—A factor used in operationally deriving the Minimal Risk Level (MRL) or Reference Dose (RfD) or Reference Concentration (RfC) from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using lowest-observed-adverse-effect level (LOAEL) data rather than no-observed-adverse-effect level (NOAEL) data. A default for each individual UF is 10; if complete certainty in data exists, a value of 1 can be used; however, a reduced UF of 3 may be used on a case-by-case basis, 3 being the approximate logarithmic average of 10 and 1.

Xenobiotic—Any chemical that is foreign to the biological system.

APPENDIX A. ATSDR MINIMAL RISK LEVELS AND WORKSHEETS

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) [42 U.S.C. 9601 et seq.], as amended by the Superfund Amendments and Reauthorization Act (SARA) [Pub. L. 99–499], requires that the Agency for Toxic Substances and Disease Registry (ATSDR) develop jointly with the U.S. Environmental Protection Agency (EPA), in order of priority, a list of hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL); prepare toxicological profiles for each substance included on the priority list of hazardous substances; and assure the initiation of a research program to fill identified data needs associated with the substances.

The toxicological profiles include an examination, summary, and interpretation of available toxicological information and epidemiologic evaluations of a hazardous substance. During the development of toxicological profiles, Minimal Risk Levels (MRLs) are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. MRLs are based on noncancer health effects only and are not based on a consideration of cancer effects. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the no-observed-adverse-effect level/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (365 days and longer) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive chemical-induced end point considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

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MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals.

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology, expert panel peer reviews, and agency-wide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published levels. For additional information regarding MRLs, please contact the Division of Toxicology, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop F-32, Atlanta, Georgia 30333.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Bromoform
CAS Number: 75-25-2
Date: August 2005
Profile Status: Final Draft of Post-Public Comment Toxicological Profile
Route: [ ] Inhalation [X] Oral
Duration: [X] Acute [ ] Intermediate [ ] Chronic
Key to Figure: 13
Species: Mice

Minimal Risk Level: 0.7 [X] mg/kg/day [ ] mg/m^3

Reference: Condie LW, Smallwood CL, Laurie RD. 1983. Comparative renal and hepatotoxicity of halomethanes: Bromodichloromethane, bromoform, chloroform, dibromochloromethane and methylene chloride. Drug Chem Toxicol 6:563-578.

Experimental design:

Groups of 5–16 male CD-1 mice received daily gavage doses of 0, 72, 145, or 289 mg/kg/day bromoform in corn oil for 14 days. Body weight was measured on days 1 and 14. Blood was collected for clinical chemistry at study termination. Renal cortical slices of kidney tissue were collected for measurement of para-aminohippurate (PAH) uptake, and samples of liver and kidney tissue were collected for histopathological examination.

Effects noted in study and corresponding concentrations:

No significant alterations in body weight gain were observed. PAH uptake by kidney slices was decreased by 30% in the 289 mg/kg/day group; a significant increase in SGPT was also observed at this dose. Minimal to moderate liver and kidney histological alterations were observed. Liver effects included centrilobular pallor at 145 and 289 mg/kg/day and focal inflammation at 289 mg/kg/day. Kidney effects consisted of epithelial hyperplasia at 289 mg/kg/day and mesangial nephrosis at 145 and 289 mg/kg/day.

Concentration and end point used for MRL derivation:

The MRL is based on a NOAEL of 72 mg/kg/day and a LOAEL of 145 mg/kg/day for hepatice centrilobular pallor in mice.

[X] NOAEL [ ] LOAEL

Uncertainty Factors used in MRL derivation:

[X] 10 for extrapolation from animals to humans
[X] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose? No.

If an inhalation study in animals, list conversion factors used in determining human equivalent concentration: NA

Was a conversion used from intermittent to continuous exposure? No.

Other additional studies or pertinent information that lend support to this MRL:

Several acute-duration studies support the identification of the liver as the most sensitive target of bromoform toxicity. The observed effects include hepatocellular vacuolization and swelling (Chu et al. 1982a; Coffin et al. 2000), centrilobular pallor (Condie et al. 1983), increased absolute and relative liver weights (Munson et al. 1982), alterations in serum chemistry enzymes such as SGPT (Munson et al. 1982), and impaired liver function (Munson et al. 1982). The highest NOAEL for liver effects is 72 mg/kg/day in mice (Condie et al. 1983); in this study, centrilobular pallor (Condie et al. 1983), which was considered to be indicative of liver degeneration, was observed at 145 mg/kg/day. At 125 mg/kg/day, increases in liver weight were observed (Munson et al. 1982) and hepatocellular vacuolization and swelling were observed at 200 mg/kg (164 mg/kg/day) (Coffin et al. 2000). Other adverse effects that have been observed at similar or higher dose levels include mesangial nephrosis at 145 mg/kg/day (NOAEL of 72 mg/kg/day) (Condie et al. 1983), impaired immune function at 125 mg/kg/day (NOAEL of 50 mg/kg/day) (Munson et al. 1982), skeletal anomalies in the offspring of rats exposed to 200 mg/kg/day (NOAEL of 100 mg/kg/day) (Ruddick et al. 1983), and central nervous system depression at ≥600 mg/kg (Balster and Borzelleca 1982; Bowman et al. 1978; NTP 1989a). Although several adverse effects have been reported at 100–200 mg/kg/day, the liver was selected as the critical target because the adverse liver effects are consistently observed in animals following acute-, intermediate-, and chronic-duration exposure.

Agency Contact (Chemical Managers): John Risher, Dennis Jones

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Bromoform
CAS Number: 75-25-2
Date: August 2005
Profile Status: Final Draft of Post-Public Comment Toxicological Profile
Route: [ ] Inhalation [X] Oral
Duration: [ ] Acute [X] Intermediate [ ] Chronic
Key to Figure: 26
Species: Rat

Minimal Risk Level: 0.2 [X] mg/kg/day [ ] mg/m^3

Reference: NTP. 1989a. Toxicology and carcinogenesis studies of tribromomethane (bromoform) in F344/N rats and B6C3F1 mice (gavage studies). National Toxicology Program. Technical Report Series No. 350. Research Triangle Park, NC: U.S. Department of Health and Human Services.

Experimental design:

Groups of F344/N male and female F344/N rats (10/sex/group) received gavage doses of 0, 12, 25, 50, 100, or 200 mg/kg bromoform in corn oil 5 days/week for 13 weeks. The rats were observed twice per day and weighed weekly. At sacrifice, all animals were necropsied and tissues from the vehicle control and high dose groups were examined histologically.

Effects noted in study and corresponding concentrations:

None of the rats died before the end of the study. Final mean body weights were similar in dosed and control groups. Lethargy was observed in all male rats exposed to 100 or 200 mg/kg and in all females exposed to 200 mg/kg. Hepatocellular vacuolization was observed in male rats (3/10, 6/10, 5/10, 8/10, 8/10, and 10/10 in the 0, 12, 25, 50, 100, and 200 mg/kg groups, respectively); the response reached statistical significance (Fisher exact one-tailed p-value of 0.03) at 50 mg/kg/day. Severity data were not reported for this lesion, but the study authors noted that vacuoles were more numerous in the 200 mg/kg group. Corresponding hepatic effects were not observed in females.

Concentration and end point used for MRL derivation:

The MRL is based on a NOAEL of 25 mg/kg (duration-adjusted to 18 mg/kg/day) and a LOAEL of 50 mg/kg (duration-adjusted to 36 mg/kg/day) for hepatic lesions (hepatocellular vacuolization).

[X] NOAEL [ ] LOAEL

Uncertainty Factors used in MRL derivation:

[X] 10 for extrapolation from animals to humans
[X] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose? No.

If an inhalation study in animals, list conversion factors used in determining human equivalent concentration: NA

Was a conversion used from intermittent to continuous exposure?

The NOAEL was adjusted for intermittent exposure: 25 mg/kg x 5 days/7 days = 18 mg/kg/day

Other additional studies or pertinent information that lend support to this MRL:

A number of animal studies have identified the liver as the critical target of bromoform oral toxicity. An intermediate-duration study in rats (Aida et al. 1992) supports the identification of 50 mg/kg as the critical dose. In this study, a LOAEL of 56.4 mg/kg/day was identified for hepatocellular vacuolization and swelling in rats exposed to bromoform in the diet for 1 month. At a higher dose (207.5 mg/kg/day), a decrease in serum triglycerides and an increase in serum cholesterol levels were found; these findings are consistent with the liver histological alterations. Mice appear to be less sensitive to the toxicity of bromoform than rats. NOAEL and LOAEL values of 100 and 200 mg/kg (5 days/week) for hepatocellular vacuolization were identified in the intermediate-duration mouse NTP study (NTP 1989a). Melnick et al. (1998) found hydropic degeneration and increases in SGPT and sorbitol dehydrogenase levels in mice receiving gavage doses of 500 mg/kg, 5 days/week for 3 weeks. Acute- and chronic-duration studies have also identified the liver as the most sensitive target of toxicity.

Agency Contact (Chemical Managers): John Risher, Dennis Jones

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Bromoform
CAS Number: 75-25-2
Date: September 2005
Profile Status: Final Draft of Post-Public Comment Toxicological Profile
Route: [ ] Inhalation [X] Oral
Duration: [ ] Acute [ ] Intermediate [X] Chronic
Key to Figure: 38
Species: Rat

Minimal Risk Level: 0.02 [X] mg/kg/day [ ] mg/m^3

Reference: NTP. 1989a. Toxicology and carcinogenesis studies of tribromomethane (bromoform) in F344/N rats and B6C3F1 mice (gavage studies). National Toxicology Program. Technical Report Series No. 350. Research Triangle Park, NC: U.S. Department of Health and Human Services.

Experimental design:

Groups of male and female F344/N rats (50/sex/group) were administered via gavage 0, 100, or 200 mg/kg bromoform in corn oil 5 days/week for 103 weeks. Animals were observed for clinical signs throughout the study. Body weights were measured weekly for 12 weeks and monthly thereafter. At termination, all study animals were necropsied. Full histopathological examination was performed on all control and high dose animals and on low dose males. Selected tissues including esophagus, gross lesions, kidney, liver, lymph nodes, mammary gland, pancreas, pituitary gland, salivary glands, thyroid gland, trachea, and uterus were examined in low-dose females.

Effects noted in study and corresponding concentrations:

Significantly increased mortality was observed in male rats exposed to 200 mg/kg after week 91 (36–78% vs. 26–32%). Survival was comparable to vehicle controls in males exposed to 100 mg/kg and in females exposed to 100 or 200 mg/kg. Bromoform-related clinical signs included lethargy in both sexes and aggressiveness in males. After 15 weeks, the difference between control body weights and body weights of males exposed to 200 mg/kg males was consistently ≥10%; terminal body weights were 21% lower than controls. In the females exposed to 200 mg/kg, the difference in body weights was ≥10% after week 41; terminal body weights were 25% lower than controls. Body weights in the 100 mg/kg groups were typically within 10% of controls. Bromoform-related hepatic lesions included fatty change (characterized as hepatocellular vacuolization) in 23/50, 49/50, and 50/50 males exposed to 0, 100, or 200 mg/kg, respectively, and 19/50, 39/49, and 46/50 females; chronic active inflammation (male: 0/50, 29/50, and 23/50; female: 9/50, 8/49, and 27/50); and necrosis (male: 7/50, 3/50, and 20/50; female: 11/50, 3/49, and 2/50). Other lesions with significantly increased incidences included salivary gland duct squamous metaplasia at 100 and 200 mg/kg (males: 0/50, 15/50, and 31/48; females: 0/49, 10/49, and 16/50) and chronic active inflammation (male: 0/50, 14/50, and 22/48; female: 0/49, 9/49, and 18/50); ulcers of the forestomach in males at 200 mg/kg (1/49, 5/50, and 10/50); chronic active inflammation in the lungs in males at 100 and 200 mg/kg (1/50, 7/50, and 15/50); squamous metaplasia in the prostate gland at 200 mg/kg (2/49, 6/46, and 12/50); hyperplasia of the anterior lobe of the pituitary gland in males at 100 mg/kg (9/48, 26/50, and 15/50); and spleen pigmentation in females at 200 mg/kg (7/49, 6/28, and 29/50), which was characteristic of hemosiderin. The occurrence of ulcers in the forestomach may have resulted from gavage bolus dose delivery. Lesions observed in the lungs and salivary glands were reported to be consistent with infection by sialodacryloadenitis (SDA) virus. However, since the

occurrence of these lesions was clearly dose-related, the study authors concluded that they were likely to represent a combination of viral and chemical-related effects.

It should be noted that the study authors did not report statistical analysis data for nonneoplastic lesions. An independent statistical analysis was conducted; statistical significance was determined using a Fisher exact test, one tailed $p<0.05$).

Concentration and end point used for MRL derivation:

The MRL is based on a LOAEL of 100 mg/kg (duration-adjusted to 71 mg/kg/day) for histopathological changes (vacuolization) in the liver.

[ ] NOAEL [X] LOAEL

Uncertainty Factors used in MRL derivation:

[X] 3 for use of a minimal LOAEL
[X] 10 for extrapolation from animals to humans
[X] 10 for human variability

Modifying Factors used in MRL derivation:

[X] 10 to account for the identification of a lower LOAEL in the 13-week NTP (1989a) study.

Was a conversion factor used from ppm in food or water to a mg/body weight dose? No.

If an inhalation study in animals, list conversion factors used in determining human equivalent concentration: NA

Was a conversion used from intermittent to continuous exposure? Yes.

The LOAEL was adjusted for intermittent exposure: 100 mg/kg x 5 days/7 days = 71 mg/kg/day

Other additional studies or pertinent information that lend support to this MRL:

Two studies have examined the chronic toxicity of bromoform (NTP 1989a; Tobe et al. 1982). Both studies identified the liver as the most sensitive target of toxicity. Both the NTP (1989a) rat and mouse studies identified LOAEL values of 100 mg/kg (5 days/week) for hepatocellular vacuolization. The rat study was selected over the mouse study because the effects were observed in both sexes. This study is supported by a 2-year dietary study conducted by Tobe et al. (1982). Yellowing of the liver and increases in absolute and liver weights were observed in female rats exposed to 140 mg/kg/day; histological examinations were not conducted. Alterations in several clinical chemistry parameters are also indicative of liver damage. Increases in SGOT and SGPT and decreases in serum triglycerides and cholesterol were observed at 590–720 mg/kg/day. A number of intermediate-duration studies (Aida et al. 1992; Chu et al. 1982b; Melnick et al. 1998; NTP 1989a) support the identification of the liver as the critical target of bromoform toxicity.

Agency Contact (Chemical Managers): John Risher, Dennis Jones

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Dibromochloromethane
CAS Number: 124-48-1
Date: August 2005
Profile Status: Final Draft of Post-Public Comment Toxicological Profile
Route: [ ] Inhalation [X] Oral
Duration: [X] Acute [ ] Intermediate [ ] Chronic
Key to Figure: 16
Species: Mouse

Minimal Risk Level: 0.1 [X] mg/kg/day [ ] mg/m^3

Reference: Condie LW, Smallwood CL, Laurie RD. 1983. Comparative renal and hepatotoxicity of halomethanes: Bromodichloromethane, bromoform, chloroform, dibromochloromethane and methylene chloride. Drug Chem Toxicol 6:563-578.

Experimental design:

Groups of 5–16 male CD-1 mice received daily gavage doses of 0, 37, 74, or 147 mg/kg/day dibromochloromethane in corn oil for 14 days. Body weight was measured on days 1 and 14. Blood was collected for clinical chemistry at study termination. Renal cortical slices of kidney tissue were collected for measurement of para-aminohippurate uptake, and samples of liver and kidney tissue were histopathological examination.

Effects noted in study and corresponding concentrations:

No deaths or treatment-related clinical signs were reported. No significant alterations in body weight gain were observed. Para-aminohippurate uptake by kidney slices was decreased by approximately 30% in the 147 mg/kg/day group; a significant increase in SGPT was also observed at this dose. Minimal to moderate liver and kidney histological alterations were observed. Liver effects included hepatocellular vacuolization at 37 mg/kg/day and higher (1/16, 3/5, 4/10, and 8/10 in the 0, 37, 74, and 147 mg/kg/day groups, respectively) and mitotic figures at 147 mg/kg/day (0/16, 0/5, 2/10, and 4/10). Kidney effects consisted of mesangial hypertrophy was observed at 37 mg/kg/day and higher (0/16, 4/5, 7/10, and 7/10).

Concentration and end point used for MRL derivation:

The MRL is based on a LOAEL of 37 mg/kg/day for hepatocellular vacuolization (Condie et al. 1983).

[ ] NOAEL [X] LOAEL

Uncertainty Factors used in MRL derivation:

[X] 3 for use of a minimal LOAEL
[X] 10 for extrapolation from animals to humans
[X] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose? No.

If an inhalation study in animals, list conversion factors used in determining human equivalent concentration: NA

Was a conversion used from intermittent to continuous exposure? No.

Other additional studies or pertinent information that lend support to this MRL:

There are numerous studies that support the identification of the liver as the critical target of dibromochloromethane toxicity. Hepatocellular vacuolization and/or swelling were observed in mice exposed to 100 and 300 mg/kg (9 doses in an 11-day period) (Coffin et al. 2000), at ≥50 mg/kg in rats and mice exposed to dibromochloromethane for an intermediate duration (Aida et al. 1992; Daniel et al. 1990; NTP 1985) and in rats and mice exposed to ≥40 for 2 years (NTP 1985). At higher doses, hepatocellular necrosis is observed (Aida et al. 1993; Daniel et al. 1990; NTP 1985) in rats and mice exposed to ≥100 mg/kg for intermediate durations.

The Condie et al. study (1983) also identified the 37 mg/kg/day dose as a LOAEL for kidney effects, mesangial cell hyperplasia. Other animal studies have also found kidney lesions following oral exposure to dibromochloromethane. Nephropathy, characterized by tubular cell degeneration and tubular cast formation was noted in 80% of male rats and 100% of female rats, but was not found in the controls or other dose groups exposed to dibromochloromethane in corn oil for 13 weeks (NTP 1985). The liver was selected as the critical target because the results of other studies suggest that it may be the more sensitive target of toxicity.

Agency Contact (Chemical Managers): John Risher, Dennis Jones

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Dibromochloromethane
CAS Number: 124-48-1
Date: August 2005
Profile Status: Final Draft of Post-Public Comment Toxicological Profile
Route: [ ] Inhalation [X] Oral
Duration: [ ] Acute [ ] Intermediate [X] Chronic
Key to Figure: 46
Species: Rat

Minimal Risk Level: 0.09 [X] mg/kg/day [ ] mg/m^3

Reference: NTP. 1985. Toxicology and carcinogenesis studies of dibromochloromethane in F344/N rats and B6C3F1 mice (gavage studies). National Toxicology Program. Technical Report Series No. 282. Research Triangle Park, NC: U.S. Department of Health and Human Services.

Experimental design:

Groups of 50 male and 50 female F344/N rats received 0, 40, or 80 mg/kg gavage doses of dibromochloromethane in corn oil 5 days/week for 2 years. Clinical signs were recorded weekly. Body weights were recorded weekly for the first twelve weeks of the study and monthly thereafter. Necropsy was performed on all animals. Histopathological examination was conducted on tissues from all dose groups.

Effects noted in study and corresponding concentrations:

Survival was comparable in all study groups. Body weight gain was within 10% of controls throughout the study. In the liver, fatty change (male: 27/50, 47/50, and 49/50; female: 12/50, 23/50, and 50/50) and "ground glass" cytoplasmic changes (male: 8/50, 22/50, and 34/50; female: 0/50, 1/50, and 12/50) were observed. A dose-related increase in nephrosis was observed in female rats (7/50, 11/50, and 14/50); however, the incidences in exposed rats was not statistically higher than in vehicle controls assessed using the Fisher exact test.

It should be noted that the study authors did not report statistical analysis data for nonneoplastic lesions. An independent statistical analysis was conducted; statistical significance was determined using a Fisher exact test, one tailed $p<0.05$).

Concentration and end point used for MRL derivation:

The MRL is based on a LOAEL of 40 mg/kg (duration-adjusted to 28 mg/kg/day) for liver histopathology (fatty change) (NTP 1985).

[ ] NOAEL [X] LOAEL

Uncertainty Factors used in MRL derivation:

[X] 3 for use of a minimal LOAEL
[X] 10 for extrapolation from animals to humans
[X] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose? No.

If an inhalation study in animals, list conversion factors used in determining human equivalent concentration: NA

Was a conversion used from intermittent to continuous exposure?

The LOAEL was adjusted for intermittent exposure: 40 mg/kg x 5 days/7 days = 28 mg/kg/day

Other additional studies or pertinent information that lend support to this MRL:

The identification of the liver as the critical target is supported by numerous acute-duration studies in rats and mice exposed to ≥37 mg/kg (Chu et al. 1982a; Coffin et al. 2000; Condie et al. 1983; Hewitt et al. 1983; Munson et al. 198; NTP 1985), 50 mg/kg for intermediate durations (Aida et al. 1992; Daniel et al. 1990; Melnick et al. 1998; NTP 1985), and 40 mg/kg for chronic durations (NTP 1985; Tobe et al. 1982). The identification of the LOAEL of 40 mg/kg for fatty changes in the liver is supported by the NTP (1985) mouse study and a rat study by Tobe et al. (1982). Fatty metamorphosis was found in mice receiving gavage doses of 50 mg/kg, 5 days/week for 2 years (NTP 1985). Necrosis was observed at 100 mg/kg. In the Tobe et al. (1982) study, groups of rats were exposed to dibromochloromethane in diet for 2 years. No histological examinations were conducted; however, hypertrophy and yellowing of the liver was found at 85 mg/kg/day. Alterations in a number of clinical chemistry parameters, which are indicative of liver damage, were also observed. Decreases in serum triglycerides were observed at 20 mg/kg/day and decreases in serum cholesterol were observed at 540 mg/kg/day.

Agency Contact (Chemical Managers): John Risher, Dennis Jones

APPENDIX B. USER'S GUIDE

Chapter 1

Public Health Statement

This chapter of the profile is a health effects summary written in non-technical language. Its intended audience is the general public, especially people living in the vicinity of a hazardous waste site or chemical release. If the Public Health Statement were removed from the rest of the document, it would still communicate to the lay public essential information about the chemical.

The major headings in the Public Health Statement are useful to find specific topics of concern. The topics are written in a question and answer format. The answer to each question includes a sentence that will direct the reader to chapters in the profile that will provide more information on the given topic.

Chapter 2

Relevance to Public Health

This chapter provides a health effects summary based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information. This summary is designed to present interpretive, weight-of-evidence discussions for human health end points by addressing the following questions:

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

The chapter covers end points in the same order that they appear within the Discussion of Health Effects by Route of Exposure section, by route (inhalation, oral, and dermal) and within route by effect. Human data are presented first, then animal data. Both are organized by duration (acute, intermediate, chronic). *In vitro* data and data from parenteral routes (intramuscular, intravenous, subcutaneous, etc.) are also considered in this chapter.

The carcinogenic potential of the profiled substance is qualitatively evaluated, when appropriate, using existing toxicokinetic, genotoxic, and carcinogenic data. ATSDR does not currently assess cancer potency or perform cancer risk assessments. Minimal Risk Levels (MRLs) for noncancer end points (if derived) and the end points from which they were derived are indicated and discussed.

Limitations to existing scientific literature that prevent a satisfactory evaluation of the relevance to public health are identified in the Chapter 3 Data Needs section.

Interpretation of Minimal Risk Levels

Where sufficient toxicologic information is available, ATSDR has derived MRLs for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not

meant to support regulatory action, but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans.

MRLs should help physicians and public health officials determine the safety of a community living near a chemical emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Chapter 2, "Relevance to Public Health," contains basic information known about the substance. Other sections such as Chapter 3 Section 3.9, "Interactions with Other Substances," and Section 3.10, "Populations that are Unusually Susceptible" provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology that the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the most sensitive end point which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen end point are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest no-observed-adverse-effect level (NOAEL) that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor (UF) of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a substance-specific MRL are provided in the footnotes of the levels of significant exposure (LSE) tables.

Chapter 3

Health Effects

Tables and Figures for Levels of Significant Exposure (LSE)

Tables and figures are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species, MRLs to humans for noncancer end points, and EPA's estimated range associated with an upper- bound individual lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. Use the LSE tables and figures for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of NOAELs, LOAELs, or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE Table 3-1 and Figure 3-1 are shown. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

LEGEND

See Sample LSE Table 3-1 (page B-6)

(1) Route of Exposure. One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. Typically when sufficient data exist, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure, i.e., inhalation, oral, and dermal (LSE Tables 3-1, 3-2, and 3-3, respectively). LSE figures are limited to the inhalation (LSE Figure 3-1) and oral (LSE Figure 3-2) routes. Not all substances will have data on each route of exposure and will not, therefore, have all five of the tables and figures.

(2) Exposure Period. Three exposure periods—acute (less than 15 days), intermediate (15–364 days), and chronic (365 days or more)—are presented within each relevant route of exposure. In this example, an inhalation study of intermediate exposure duration is reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Health Effect. The major categories of health effects included in LSE tables and figures are death, systemic, immunological, neurological, developmental, reproductive, and cancer. NOAELs and LOAELs can be reported in the tables and figures for all effects but cancer. Systemic effects are further defined in the "System" column of the LSE table (see key number 18).

(4) Key to Figure. Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 18 has been used to derive a NOAEL and a Less Serious LOAEL (also see the two "18r" data points in sample Figure 3-1).

(5) Species. The test species, whether animal or human, are identified in this column. Chapter 2, "Relevance to Public Health," covers the relevance of animal data to human toxicity and Section 3.4, "Toxicokinetics," contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(6) Exposure Frequency/Duration. The duration of the study and the weekly and daily exposure regimens are provided in this column. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 18), rats were exposed to “Chemical x” via inhalation for 6 hours/day, 5 days/week, for 13 weeks. For a more complete review of the dosing regimen, refer to the appropriate sections of the text or the original reference paper (i.e., Nitschke et al. 1981).

(7) System. This column further defines the systemic effects. These systems include respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, and dermal/ocular. "Other" refers to any systemic effect (e.g., a decrease in body weight) not covered in these systems. In the example of key number 18, one systemic effect (respiratory) was investigated.

(8) NOAEL. A NOAEL is the highest exposure level at which no harmful effects were seen in the organ system studied. Key number 18 reports a NOAEL of 3 ppm for the respiratory system, which was used to derive an intermediate exposure, inhalation MRL of 0.005 ppm (see footnote "b").

(9) LOAEL. A LOAEL is the lowest dose used in the study that caused a harmful health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific end point used to quantify the adverse effect accompanies the LOAEL. The respiratory effect reported in key number 18 (hyperplasia) is a Less Serious LOAEL of 10 ppm. MRLs are not derived from Serious LOAELs.

(10) Reference. The complete reference citation is given in Chapter 9 of the profile.

(11) CEL. A CEL is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases.

(12) Footnotes. Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. Footnote "b" indicates that the NOAEL of 3 ppm in key number 18 was used to derive an MRL of 0.005 ppm.

LEGEND

See Sample Figure 3-1 (page B-7)

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(13) Exposure Period. The same exposure periods appear as in the LSE table. In this example, health effects observed within the acute and intermediate exposure periods are illustrated.

(14) Health Effect. These are the categories of health effects for which reliable quantitative data exists. The same health effects appear in the LSE table.

(15) Levels of Exposure. Concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(16) NOAEL. In this example, the open circle designated 18r identifies a NOAEL critical end point in the rat upon which an intermediate inhalation exposure MRL is based. The key number 18 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 3 ppm (see entry 18 in the table) to the MRL of 0.005 ppm (see footnote "b" in the LSE table).

(17) CEL. Key number 38m is one of three studies for which CELs were derived. The diamond symbol refers to a CEL for the test species-mouse. The number 38 corresponds to the entry in the LSE table.

(18) Estimated Upper-Bound Human Cancer Risk Levels. This is the range associated with the upper-bound for lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. These risk levels are derived from the EPA's Human Health Assessment Group's upper-bound estimates of the slope of the cancer dose response curve at low dose levels (q_1*).

(19) Key to LSE Figure. The Key explains the abbreviations and symbols used in the figure.

SAMPLE

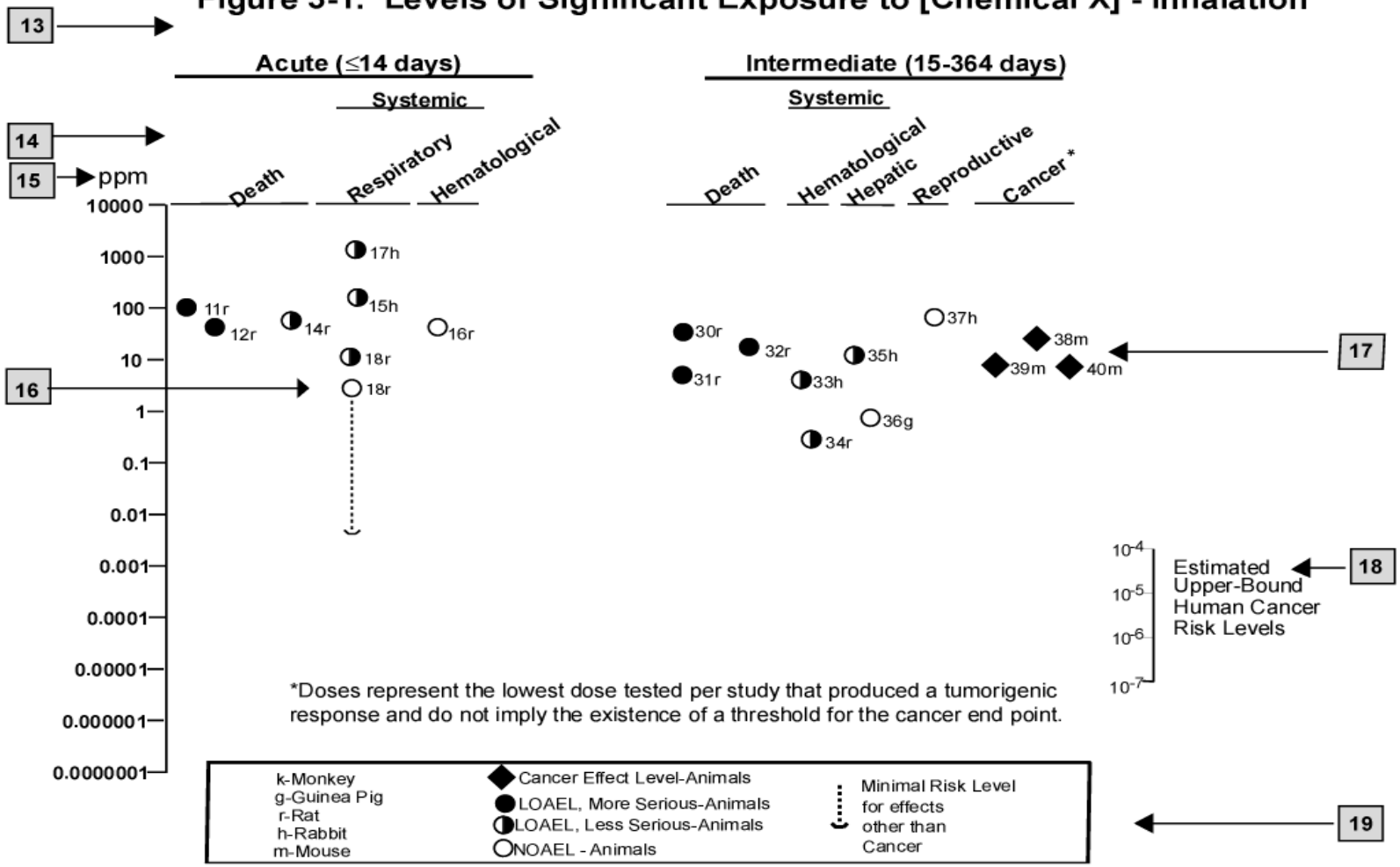
1 → **Table 3-1. Levels of Significant Exposure to [Chemical x] – Inhalation**

	Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
2 →	INTERMEDIATE EXPOSURE							
		5	6	7	8	9		10
3 →	Systemic	↓	↓	↓	↓	↓		↓
4 →	18	Rat	13 wk 5 d/wk 6 hr/d	Resp	3[b]	10 (hyperplasia)		Nitschke et al. 1981
	CHRONIC EXPOSURE							
	Cancer						11	
							↓	
	38	Rat	18 mo 5 d/wk 7 hr/d				20 (CEL, multiple organs)	Wong et al. 1982
	39	Rat	89–104 wk 5 d/wk 6 hr/d				10 (CEL, lung tumors, nasal tumors)	NTP 1982
	40	Mouse	79–103 wk 5 d/wk 6 hr/d				10 (CEL, lung tumors, hemangiosarcomas)	NTP 1982

12 → [a] The number corresponds to entries in Figure 3-1.
[b] Used to derive an intermediate inhalation Minimal Risk Level (MRL) of 5×10^{-3} ppm; dose adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animal to humans, 10 for human variability).

SAMPLE

Figure 3-1. Levels of Significant Exposure to [Chemical X] - Inhalation



APPENDIX C. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
ACOEM	American College of Occupational and Environmental Medicine
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
AED	atomic emission detection
AFID	alkali flame ionization detector
AFOSH	Air Force Office of Safety and Health
ALT	alanine aminotransferase (also known as SGPT)
AML	acute myeloid leukemia
AOAC	Association of Official Analytical Chemists
AOEC	Association of Occupational and Environmental Clinics
AP	alkaline phosphatase
APHA	American Public Health Association
AST	aspartate aminotranferase (also known as SGOT)
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AWQC	Ambient Water Quality Criteria
BAT	best available technology
BCF	bioconcentration factor
BEI	Biological Exposure Index
BMD	benchmark dose
BMR	benchmark response
BSC	Board of Scientific Counselors
C	centigrade
CAA	Clean Air Act
CAG	Cancer Assessment Group of the U.S. Environmental Protection Agency
CAS	Chemical Abstract Services
CDC	Centers for Disease Control and Prevention
CEL	cancer effect level
CELDS	Computer-Environmental Legislative Data System
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
Ci	curie
CI	confidence interval
CL	ceiling limit value
CLP	Contract Laboratory Program
cm	centimeter
CML	chronic myeloid leukemia
CPSC	Consumer Products Safety Commission
CWA	Clean Water Act
DHEW	Department of Health, Education, and Welfare
DHHS	Department of Health and Human Services
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	Department of Energy
DOL	Department of Labor

DOT	Department of Transportation
DOT/UN/NA/IMCO	Department of Transportation/United Nations/North America/International Maritime Dangerous Goods Code
DWEL	drinking water exposure level
ECD	electron capture detection
ECG/EKG	electrocardiogram
EEG	electroencephalogram
EEGL	Emergency Exposure Guidance Level
EPA	Environmental Protection Agency
F	Fahrenheit
F_1	first-filial generation
FAO	Food and Agricultural Organization of the United Nations
FDA	Food and Drug Administration
FEMA	Federal Emergency Management Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FPD	flame photometric detection
fpm	feet per minute
FR	Federal Register
FSH	follicle stimulating hormone
g	gram
GC	gas chromatography
gd	gestational day
GLC	gas liquid chromatography
GPC	gel permeation chromatography
HPLC	high-performance liquid chromatography
HRGC	high resolution gas chromatography
HSDB	Hazardous Substance Data Bank
IARC	International Agency for Research on Cancer
IDLH	immediately dangerous to life and health
ILO	International Labor Organization
IRIS	Integrated Risk Information System
Kd	adsorption ratio
kg	kilogram
kkg	metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{50}	lethal concentration, 50% kill
LC_{Lo}	lethal concentration, low
LD_{50}	lethal dose, 50% kill
LD_{Lo}	lethal dose, low
LDH	lactic dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
LT_{50}	lethal time, 50% kill
m	meter
MA	*trans,trans*-muconic acid
MAL	maximum allowable level
mCi	millicurie

MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
MFO	mixed function oxidase
mg	milligram
mL	milliliter
mm	millimeter
mmHg	millimeters of mercury
mmol	millimole
mppcf	millions of particles per cubic foot
MRL	Minimal Risk Level
MS	mass spectrometry
NAAQS	National Ambient Air Quality Standard
NAS	National Academy of Science
NATICH	National Air Toxics Information Clearinghouse
NATO	North Atlantic Treaty Organization
NCE	normochromatic erythrocytes
NCEH	National Center for Environmental Health
NCI	National Cancer Institute
ND	not detected
NFPA	National Fire Protection Association
ng	nanogram
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NIOSHTIC	NIOSH's Computerized Information Retrieval System
NLM	National Library of Medicine
nm	nanometer
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NOES	National Occupational Exposure Survey
NOHS	National Occupational Hazard Survey
NPD	nitrogen phosphorus detection
NPDES	National Pollutant Discharge Elimination System
NPL	National Priorities List
NR	not reported
NRC	National Research Council
NS	not specified
NSPS	New Source Performance Standards
NTIS	National Technical Information Service
NTP	National Toxicology Program
ODW	Office of Drinking Water, EPA
OERR	Office of Emergency and Remedial Response, EPA
OHM/TADS	Oil and Hazardous Materials/Technical Assistance Data System
OPP	Office of Pesticide Programs, EPA
OPPT	Office of Pollution Prevention and Toxics, EPA
OPPTS	Office of Prevention, Pesticides and Toxic Substances, EPA
OR	odds ratio
OSHA	Occupational Safety and Health Administration
OSW	Office of Solid Waste, EPA
OTS	Office of Toxic Substances

OW	Office of Water
OWRS	Office of Water Regulations and Standards, EPA
PAH	polycyclic aromatic hydrocarbon
PBPD	physiologically based pharmacodynamic
PBPK	physiologically based pharmacokinetic
PCE	polychromatic erythrocytes
PEL	permissible exposure limit
pg	picogram
PHS	Public Health Service
PID	photo ionization detector
pmol	picomole
PMR	proportionate mortality ratio
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
PSNS	pretreatment standards for new sources
RBC	red blood cell
REL	recommended exposure level/limit
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
RQ	reportable quantity
RTECS	Registry of Toxic Effects of Chemical Substances
SARA	Superfund Amendments and Reauthorization Act
SCE	sister chromatid exchange
SGOT	serum glutamic oxaloacetic transaminase (also known as AST)
SGPT	serum glutamic pyruvic transaminase (also known as ALT)
SIC	standard industrial classification
SIM	selected ion monitoring
SMCL	secondary maximum contaminant level
SMR	standardized mortality ratio
SNARL	suggested no adverse response level
SPEGL	Short-Term Public Emergency Guidance Level
STEL	short term exposure limit
STORET	Storage and Retrieval
TD_{50}	toxic dose, 50% specific toxic effect
TLV	threshold limit value
TOC	total organic carbon
TPQ	threshold planning quantity
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TWA	time-weighted average
UF	uncertainty factor
U.S.	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey
VOC	volatile organic compound
WBC	white blood cell
WHO	World Health Organization

>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to
%	percent
α	alpha
β	beta
γ	gamma
δ	delta
µm	micrometer
µg	microgram
q_1^*	cancer slope factor
–	negative
+	positive
(+)	weakly positive result
(–)	weakly negative result

APPENDIX D. INDEX

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TOXICOLOGICAL PROFILE FOR BROMOMETHANE

Agency for Toxic Substances and Disease Registry
U.S. Public Health Service

September 1992

DISCLAIMER

The use of company or product name(s) is for identification only and does not imply endorsement by the Agency for Toxic Substances and Disease Registry.

FOREWORD

The Superfund Amendments and Reauthorization Act (SARA) of 1986 (Public Law 99-499) extended and amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). This public law directed the Agency for Toxic Substances and Disease Registry (ATSDR) to prepare toxicological profiles for hazardous substances which are most commonly found at facilities on the CERCLA National Priorities List and which pose the most significant potential threat to human health, as determined by ATSDR and the Environmental Protection Agency (EPA). The lists of the 250 most significant hazardous substances were published in the Federal Register on April 17, 1987; on October 20, 1988; on October 26, 1989; and on October 17, 1990. A revised list of 275 substances was published on October 17, 1991.

Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the lists. Each profile must include the following content:

(A) An examination, summary, and interpretation of available toxicological information and epidemiological evaluations on the hazardous substance in order to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects.

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure which present a significant risk to human health of acute, subacute, and chronic health effects.

(C) Where appropriate, an identification of toxicological testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

This toxicological profile is prepared in accordance with guidelines developed by ATSDR and EPA. The original guidelines were published in the Federal Register on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile is intended to characterize succinctly the toxicological and adverse health effects information for the hazardous substance being described. Each profile identifies and reviews the key literature (that has been peer-reviewed) that describes a hazardous substance's toxicological properties. Other pertinent literature is also presented but described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

Foreword

Each toxicological profile begins with a public health statement, which describes in nontechnical language a substance's relevant toxicological properties. Following the public health statement is information concerning levels of significant human exposure and, where known, significant health effects. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to protection of public health will be identified by ATSDR, the National Toxicology Program (NTP) of the Public Health Service, and EPA. The focus of the profiles is on health and toxicological information; therefore, we have included this information in the beginning of the document.

The principal audiences for the toxicological profiles are health professionals at the federal, state, and local levels, interested private sector organizations and groups, and members of the public.

This profile reflects our assessment of all relevant toxicological testing and information that has been peer reviewed. It has been reviewed by scientists from ATSDR, the Centers for Disease Control, the NTP, and other federal agencies. It has also been reviewed by a panel of nongovernment peer reviewers. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

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1. PUBLIC HEALTH STATEMENT

This Statement was prepared to give you information about bromomethane and to emphasize the human health effects that may result from exposure to it. The Environmental Protection Agency (EPA) has identified 1,177 sites on its National Priorities List (NPL). Bromomethane has been found in at least 12 of these sites. However, we do not know how many of the 1,177 NPL sites have been evaluated for bromomethane. As EPA evaluates more sites, the number of sites at which bromomethane is found may change. This information is important for you to know because bromomethane may cause harmful health effects and because these sites are potential or actual sources of human exposure to bromomethane.

When a chemical is released from a large area, such as an industrial plant, or from a container, such as a drum or bottle, it enters the environment as a chemical emission. This emission, which is also called a release, does not always lead to exposure. You can be exposed to a chemical only when you come into contact with the chemical. You may be exposed to it in the environment by breathing, eating, or drinking substances containing the chemical or from skin contact with it.

If you are exposed to a hazardous chemical such as bromomethane, several factors will determine whether harmful health effects will occur and what the type and severity of those health effects will be. These factors include the dose (how much), the duration (how long), the route or pathway by which you are exposed (breathing, eating, drinking, or skin contact), the other chemicals to which you are exposed, and your individual characteristics such as age, sex, nutritional status, family traits, life style, and state of health.

1.1 WHAT IS BROMOMETHANE?

Bromomethane (also called methyl bromide) is a colorless gas without much smell. Some bromomethane is formed in the ocean, probably by algae or kelp. However, most is made by humans to kill various pests (rats, insects, fungus, etc.) that might be present in homes, foods, or soil. Some bromomethane is also used to make other chemicals.

Bromomethane is usually stored in sealed containers to keep it from evaporating. If leaking containers of bromomethane are put in a waste site, most of the bromomethane will probably escape into the air. Small amounts might leak into the soil or pass through the soil and dissolve in underground water. Bromomethane has been found in underground water at two hazardous waste sites on the NPL.

Bromomethane breaks down in the environment to other chemicals. In air, it usually takes about 11 months for half the bromomethane that was released to disappear. In underground water, it usually takes about 1 month for half the bromomethane to break down.

More information on the properties and use of bromomethane and how it behaves in the environment may be found in Chapters 3, 4, and 5.

1.2 HOW MIGHT I BE EXPOSED TO BROMOMETHANE?

Because bromomethane is a gas, you are most likely to be exposed by breathing it in air. In most places around the world, levels in air are usually less than 0.025 parts of bromomethane per billion parts of air (ppb). Some cities have higher levels (up to about l-2 ppb) because of releases from chemical factories and automobile exhausts. You will probably not be exposed to high levels unless you are near a place where bromomethane is being used for fumigation. Workers who fumigate homes or fields may be exposed to very high levels if proper safety precautions are not followed. Because bromomethane evaporates so quickly, it is usually not found in food, surface water, or soil.

More information on how you might be exposed to bromomethane is given in Chapter 5.

1.3 HOW CAN BROMOMETHANE ENTER AND LEAVE MY BODY?

If bromomethane is present at a waste site, you are most likely to be exposed to it by breathing the vapors in contaminated air. You might also be exposed by drinking water from contaminated wells, although this is less likely. If you breathe in bromomethane, about half of it will pass through your lungs and enter your blood. Studies in animals suggest that if you swallow bromomethane in water, nearly all of it will pass through your stomach or intestines and enter your body. Bromomethane that enters your body either from your lungs or stomach is quickly spread throughout your body by your blood. Most bromomethane in your body is broken down into other chemicals, and these chemicals leave your body in the urine or in the air you breathe out. This usually begins happening within minutes, and is usually nearly complete within several days. We do not know how much bromomethane can enter your body through the skin, but the amount is probably small.

More information on how bromomethane enters and leaves your body is given in Chapter 2.

1.4 HOW CAN BROMOMETHANE AFFECT MY HEALTH?

If you breathe bromomethane, you may develop a headache and begin to feel weak and nauseated several hours later. If you breathe a large amount, fluid may build up in your lungs and it may be hard to breathe. You may have muscle tremors, and sometimes even seizures. Your kidneys may also be injured, and urine production may slow or stop. In severe cases, these effects can lead to death. In less serious cases, most of these effects usually disappear after several weeks, but some of the effects may never go away.

Studies in animals suggest that if you swallow bromomethane, you might experience stomach irritation but would probably not experience lung, kidney, or brain injury. Bromomethane that gets on your skin can cause itching, redness, and blisters.

Studies in animals also suggest that bromomethane does not cause birth defects and does not interfere with normal reproduction except at high exposure levels. Animals that breathed bromomethane for 2 years did not develop cancer. Animals that swallowed bromomethane for 25 weeks had changes in their stomachs that could have been an early sign of cancer, but we do not know if swallowing bromomethane for a longer time would cause cancer. Both the International Agency for Research on Cancer and the EPA have determined that bromomethane is not classifiable as to its carcinogenicity in humans.

More information on the health effects of bromomethane in humans and animals can be found in Chapter 2.

1.5 IS THERE A MEDICAL TEST TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO BROMOMETHANE?

Several tests are available to tell whether you have been exposed to bromomethane, but ‘each has limitations. The most direct test measures bromomethane in your blood or in the air you breathe out. However, this test is not usually used because most bromomethane does not stay in the body very long (see Section 1.3) and special measuring equipment is needed. More often, the main breakdown product of bromomethane (bromide) is measured in blood samples. Bromide is normally present in the blood of all people, but the levels of bromide increase when people are exposed to bromomethane. The amount of increase depends on the level of exposure. Tests for bromide are only useful if done within 1-2 days following exposure, and are not very helpful in predicting if exposed persons will have health effects or how serious the effects will be, because not all people respond to bromomethane the same way.

More information on how bromomethane can be measured in exposed humans is presented in Chapters 2 and 6.

1.6 WHAT RECOMMENDATIONS HAS THE FEDERAL GOVERNMENT MADE TO PROTECT HUMAN HEALTH?

Concentrated bromomethane can be very dangerous, so the EPA allows only licensed professional fumigators to buy or use bromomethane. The government does not have any regulations at present about how much bromomethane can be present in outdoor air or water, but EPA requires water companies to test for this chemical in their water. The Food and Drug Administration (FDA) has set limits of 125-400 parts of bromide per million parts of food (ppm) for how much bromide may remain in food after the food is treated with bromomethane. The Occupational Safety and Health Agency (OSHA) limits the average level of bromomethane in workplace air to 5 ppm, and recommends that exposures be reduced to the lowest level feasible.

More information on government rules regarding bromomethane can be found in Chapter 7.

1.7 WHERE CAN I GET MORE INFORMATION?

If you have any more questions or concerns not covered here, please contact your state health or environmental department or:

Agency for Toxic Substances and Disease Registry
Division of Toxicology
1600 Clifton Road, E-29
Atlanta, Georgia '30333

This agency can also provide you with information on the location of the nearest occupational and environmental health clinic. Such clinics specialize in recognizing, evaluating, and treating illnesses that result from exposure to hazardous substances.

2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective of the toxicology of bromomethane and a depiction of significant exposure levels associated with various adverse health effects. It contains descriptions and evaluations of studies and presents levels of significant exposure for bromomethane based on toxicological studies and epidemiological investigations.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure--inhalation, oral, and dermal--and then by health effect--death, systemic, immunological, neurological, developmental, reproductive, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods--acute (14 days or less), intermediate (15-364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. These distinctions are intended to help the users of the document identify the levels of exposure at which adverse health effects start to appear. They should also help to determine whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the tables and figures may differ depending on the user's perspective. For example, physicians concerned with the interpretation of clinical findings in exposed persons may be interested in levels of exposure associated with "serious" effects. Public health officials and project managers concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAEL) or exposure levels below which no adverse effects (NOAEL) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels, MRLs) may be of interest to health professionals and citizens alike.

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made, where data were believed reliable, for the most sensitive noncancer effect for each exposure duration. MRLs include adjustments to reflect human variability from laboratory animal data to humans.

Although methods have been established to derive these levels (Barnes et al. 1988; EPA 1989d), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

2.2.1 Inhalation Exposure

Bromomethane exists as a gas under ordinary temperature and pressure, so most studies of bromomethane toxicity have focused on the inhalation route of exposure. Studies with reliable quantitative exposure-response information are summarized in Table 2-l and Figure 2-1, and the adverse effects of inhalation exposure are discussed below.

2.2.1.1 Death

There are many reports of humans who have died following acute inhalation exposure to bromomethane. Most cases have involved accidental exposures associated with manufacturing or packaging operations, use of fire extinguishers containing bromomethane, or fumigation activities (Alexeeff and Kilgore 1983). Death is not immediate, but usually occurs within l-2 days of exposure (Marraccini et al. 1983; Prain and Smith 1952). The cause of death is not certain, but is probably due to neurological and lung injury. Fatal exposure levels in humans are usually not known, but limited data suggest the value depends in part on exposure duration. For example, lethality has been reported in humans following exposure to 60,000 ppm for 2 hours (Wyers 1945) and 1,600-8,000 ppm for 4-6 hours (Holling and Clarke 1944; Miller 1943).

Studies in animals indicate that acute exposures to levels of 160-980 ppm may be lethal (Alexeeff et al. 1985; Eustis et al. 1988; Honma et al. 1985; Hurtt et al. 1987a; Irish et al. 1940; Kato et al. 1986). Several studies reveal there is an extremely narrow margin between lethal and nonlethal exposures. For example, Kato et al. (1986) found no deaths in rats exposed to 700 ppm for 4 hours, but 100% lethality in animals exposed to 800 ppm. Similarly, Irish et al. (1940) found 100% survival in rats exposed to 100 ppm for 24 hours, and 100% lethality at 220 ppm. Longer-term exposures of animals can lead to death after exposure to levels as low as 66-120 ppm (Drew 1984; Haber 1987; Hardin et al. 1981; Irish et al. 1940; Reuzel et al. 1987).

The highest NOAEL values and all reliable LOAEL values for lethality in each species and duration category are recorded in Table 2-l and plotted in Figure 2-l.

TABLE 2-1. Levels of Significant Exposure to Bromomethane - Inhalation

Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
ACUTE EXPOSURE							
Death							
1	Human	2 hr				60000 (death in 1 hour)	Wyers 1945
2	Human	6 hr				8200 (death from pulmonary edema)	Miller 1943
3	Human	4 hr				1600 (3 men died)	Holling and Clarke 1944
4	Rat	5 d 6hr/d		250		325 (3/5 died)	Hurtt et al. 1987a
5	Rat	22 hr (1 exp)		110		260 (LC100)	Irish et al. 1940
6	Rat	8 hr (1 exp)				302 (LC50 -- 8 hours)	Honma et al. 1985
7	Rat	4 hr		700		780 (LC50)	Kato et al. 1986
8	Mouse	2 wk 5d/wk 6hr/d				160 (50% lethality in 8-10 days)	Eustis et al. 1988
9	Mouse	1 hr (1 exp)		900		980 (1/6 died) 1160 (LC50)	Alexeeff et al. 1985
Systemic							
10	Rat	5 d 6hr/d	Resp	90	175 (mild injury to nasal epithelium)	325 (severe injury to nasal epithelium)	Hurtt et al. 1987a
			Hepatic	250	325 (focal necrosis)		
			Renal	325			
			Other	90	175 (vacuolization, lipid accumulation in renal cortex)		
11	Rat	1-5 d 6hr/d	Resp	90		200 (loss of olfactory epithelium)	Hurtt et al. 1988a

Table 2-1 (Continued)

Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
12	Mouse	2 wk 5d/wk 6hr/d	Resp			160 (degeneration of nasal epithelium)	Eustis et al. 1988
			Cardio			160 (cardiomyopathy)	
			Hemato		160 (decreased RBC, increased WBC)		
			Renal			160 (nephrosis)	
13	Mouse	1 hr (1 exp)	Resp	440	560 (decreased lung weight)		Alexeeff et al. 1985
			Gastro	1200		1490 (colon hemorrhage)	
			Hepatic	440	560 (decreased liver weight)	1200 (congestion, hemorrhage)	
			Renal	700		900 (enlarged, pale kidney)	
Neurological							
14	Human	1-2 wk (occup)				100 (impaired vision, ataxia, numbness)	Johnstone 1945
15	Rat	8 hr (1 exp)		16[b]	31 (decreased brain neurotrans-mitters)		Honma 1987
16	Rat	5 d 6hr/d		175		250 (ataxia, CNS necrosis)	Hurtt et al. 1987a
17	Rat	8 hr (1 exp)				63 (impaired reflexes)	Honma et al. 1985
18	Rat	8 hr (1 exp)		31	63 (altered neuro-transmitter levels)		Honma et al.1987
19	Rabbit	13 d Gd 7-19 6hr/d		40	80 (ataxia, lethargy)		Breslin et al. 1990
20	Mouse	1 hr (1 exp)		560	700 (hyperactivity)	980 (cerebral hemorrhage, ataxia, tremors)	Alexeeff et al. 1985
21	Mouse	2 wk 5d/wk 6hr/d				160 (necrosis in CNS)	Eustis et al. 1988

Table 2-1 (Continued)

Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
Developmental							
22	Rabbit	13 d Gd 7-19 6hr/d		40		80 (increased anomalies and malformations)	Breslin et al. 1990
Reproductive							
23	Rat	5 d 6hr/d		200			Hurtt and Working 1988
24	Rat	5 d 6hr/d		250	325 (delayed spermiation)		Hurtt et al. 1987a
25	Mouse	2 wk 5d/wk 6hr/d				160 (testicular degeneration)	Eustis et al. 1988
INTERMEDIATE EXPOSURE							
Death							
26	Rat	36 wk 5d/wk 6hr/d		55			Anger et al. 1981
27	Rat	3-6 wk 5d/wk 6hr/d				160 (50% lethality after 3 weeks)	Eustis et al. 1988
28	Rat	3 wk 5d/wk 4hr/d		200		300 (3/12 died)	Ikeda et al. 1980
29	Rat	6 mo 5d/wk 8hr/d		66		100	Irish et al. 1940
30	Rabbit	15 d Gd1-15 6hr/d		20		70 (24/25 died)	Hardin et al. 1981
31	Rabbit	6 mo 5d/wk 8hr/d		33		66 (14/42 died)	Irish et al. 1940

Table 2-1 (Continued)

Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
32	Gn Pig	6 mo 5d/wk 8hr/d		66		100 (4/11 died)	Irish et al. 1940
33	Mouse	20 wk 5d/wk 6hr/d				100 (48% of males died)	Haber 1987
34	Mouse	13 wk 5d/wk 6hr/d		80		120 (increased mortality)	Drew 1984
Systemic							
35	Rat	6 mo 5d/wk 8hr/d	Resp	66	100 (mild congestion)		Irish et al. 1940
36	Rat	6 wk 5d/wk 4hr/d	Resp		300 (minor lesions)		Kato et al. 1986
			Cardio		150 (focal fibrosis)	300 (increased serum level of cardiac enzymes)	
			Hemato	400			
			Hepatic		300 (fatty degeneration)		
			Renal	400			
			Other	150	200 (decreased body weight)		
37	Rat	3-6 wk 5d/wk 6hr/d	Resp			160 (degeneration of nasal epithelium)	Eustis et al. 1988
			Cardio			160 (cardiomyopathy)	
			Hepatic		160 (minimal necrosis)		
			Renal		160 (minimal nephrosis)		
38	Rabbit	6 mo 5d/wk 8hr/d	Resp	17		33 (congestion, pneumonia)	Irish et al. 1940
			Cardio	66			
			Hepatic	66			
			Renal	66			

Table 2-1 (Continued)

Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
39	Gn Pig	6 mo 5d/wk 8hr/d	Resp Hepatic Renal	100 100 100			Irish et al. 1940
40	Mouse	20 wk 5d/wk 6hr/d	Hemato Other	100		100 (severe body weight loss)	Haber 1987
41	Mouse	13 wk 5d/wk 6hr/d	Hemato	120			Drew 1984
Neurological							
42	Rat	36 wk 5d/wk 6hr/d		55			Anger et al. 1981
43	Rat	6 wk 5d/wk 4hr/d		200		300 (paralysis)	Kato et al. 1986
44	Rat	3 wk (cont)		5[c]	10 (decreased neuro-transmitters)		Honma et al.1982
45	Rat	3 wk 5d/wk 4hr/d			200 (altered behavior)		Ikeda et al. 1980
46	Rat	3-6 wk 5d/wk 6hr/d				160 (CNS necrosis)	Eustis et al. 1988
47	Rat	4 wks 4d/wk 7.5hr/d		65			Anger et al. 1981
48	Rabbit	6 mo 5d/wk 8hr/d		17		33 (paralysis)	Irish et al. 1940
49	Rabbit	4 wks 4d/wk 7.5hr/d				65 (impaired nerve function)	Anger et al. 1981
50	Rabbit	8 mo 4d/wk 7.5hr/d		27			Russo et al. 1984

Table 2-1 (Continued)

Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
51	Mouse	13 wk 5d/wk 6hr/d		40	80 (mild limb crossing and twitching)	120 (severe limb crossing and twitching)	Drew 1984
52	Mouse	20 wk 5d/wk 6hr/d				100 (tremors, paralysis)	Haber 1987
53	Monkey	6 mo 5d/wk 8hr/d		33		66 (convulsions)	Irish et al. 1940
Developmental							
54	Rat	6 wk 5d/wk 7hr/d		70			Sikov et al. 1980
55	Rat	19 d Gd1-19 6hr/d		70			Hardin et al. 1981
56	Rabbit	24 d 7hr/d		20			Sikov et al. 1980
57	Rabbit	15 d Gd1-15 6hr/d		70			Hardin et al. 1981
Reproductive							
58	Rat	3-6 wk 5d/wk 6hr/d				160 (testicular degeneration)	Eustis et al. 1988
59	Rat	6 wk 5d/wk 7hr/d		70			Sikov et al. 1980
60	Rat	19 d Gd1-19 6hr/d		70			Hardin et al. 1981
61	Rat	6 wk 5d/wk 4hr/d		300		400 (testicular atrophy)	Kato et al. 1986

Table 2-1 (Continued)

Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
62	Rabbit	24 d 7hr/d		20			Sikov et al. 1980
63	Mouse	13 wk 5d/wk 6hr/d		80	120 (decreased sperm density)		Drew 1984
CHRONIC EXPOSURE							
Death							
64	Rat	128 wk 5d/wk 6hr/d				90 (early mortality)	Reuzel et al. 1987
Systemic							
65	Rat	128 wk 5d/wk 6hr/d	Resp		3 (irritation of nasal epithelium)	90 (hemothorax)	Reuzel et al. 1987
			Cardio	30		90 (thrombi in heart, myocardial degeneration)	
			Gastro	30	90 (hyperkeratosis of esophagus)		
			Hemato	90			
			Renal	90			
66	Mouse	103 wk 5d/wk 6hr/d	Hemato	33			Haber 1987
Neurological							
67	Human	8 yr (occup)			2.3[d] (increased prevalence of muscle ache, fatigue, ataxia)		Anger et al. 1986
68	Mouse	103 wk 5d/wk 6hr/d		10	33 (abnormal posture)		Haber 1987

Table 2-1 (Continued)

Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
Developmental							
69	Rat	2 gen. 5d/wk 6hr/d		3	30 (reduced pup weights)		Enloe et al. 1986

[a]The number corresponds to entries in Figure 2-1.
[b]Used to derive acute inhalation Minimal Risk Level (MRL) of 0.05 ppm (50 ppb); animal dose extrapolated to human dose according to method of EPA (1989d); values of blood/air partition coefficients assumed to be equal for animals and humans; dose adjusted for less-than-continuous exposure (8 hours/24 hours), and divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans, and 10 for human variability).
[c]Used to derive intermediate MRL of 0.05 ppm (50 ppb); animal dose extrapolated to human dose according to method of EPA (1989d); values of blood/air partition coefficients assumed to be equal for animals and humans; dose value divided by an uncertainty factor of 100 (10 for extrapolation from humans to animals, and 10 for human variability).
[d]Used to derive chronic MRL of 0.005 ppm (5 ppb); dose adjusted for intermittant exposure (8 hours/day, 5 days/wk), and divided by an uncertainty factor of 100 (10 for use of a LOAEL, and 10 for human variability).

Cardio = cardiovascular; CNS = central nervous system; cont = continuous; d = day(s); exp = exposure; Gastro = gastrointestinal; Gd = gestation day; gen. = generation; Gn pig = guinea pig; Hemato = hematological; hr = hour(s); LC50 = lethal concentration, 50% kill; LOAEL = lowest-observed-adverse-effect level; mo = month; NOAEL = no-observed-adverse-effect level; occup = occupational; RBC = red blood cell; Resp = respiratory; WBC = white blood cell; wk = week(s); yr = year(s)

FIGURE 2-1. Levels of Significant Exposure to Bromomethane – Inhalation

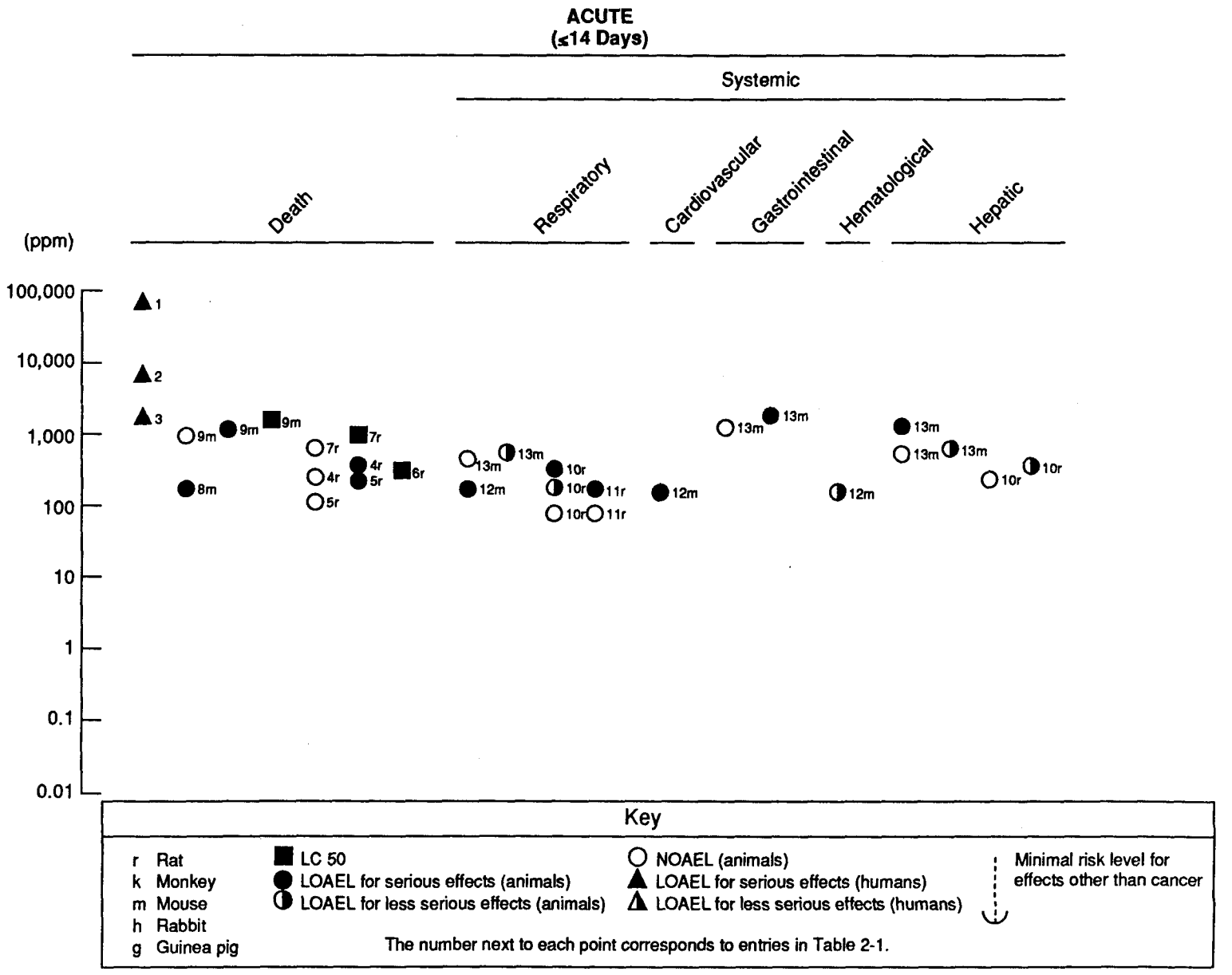


FIGURE 2-1 (Continued)

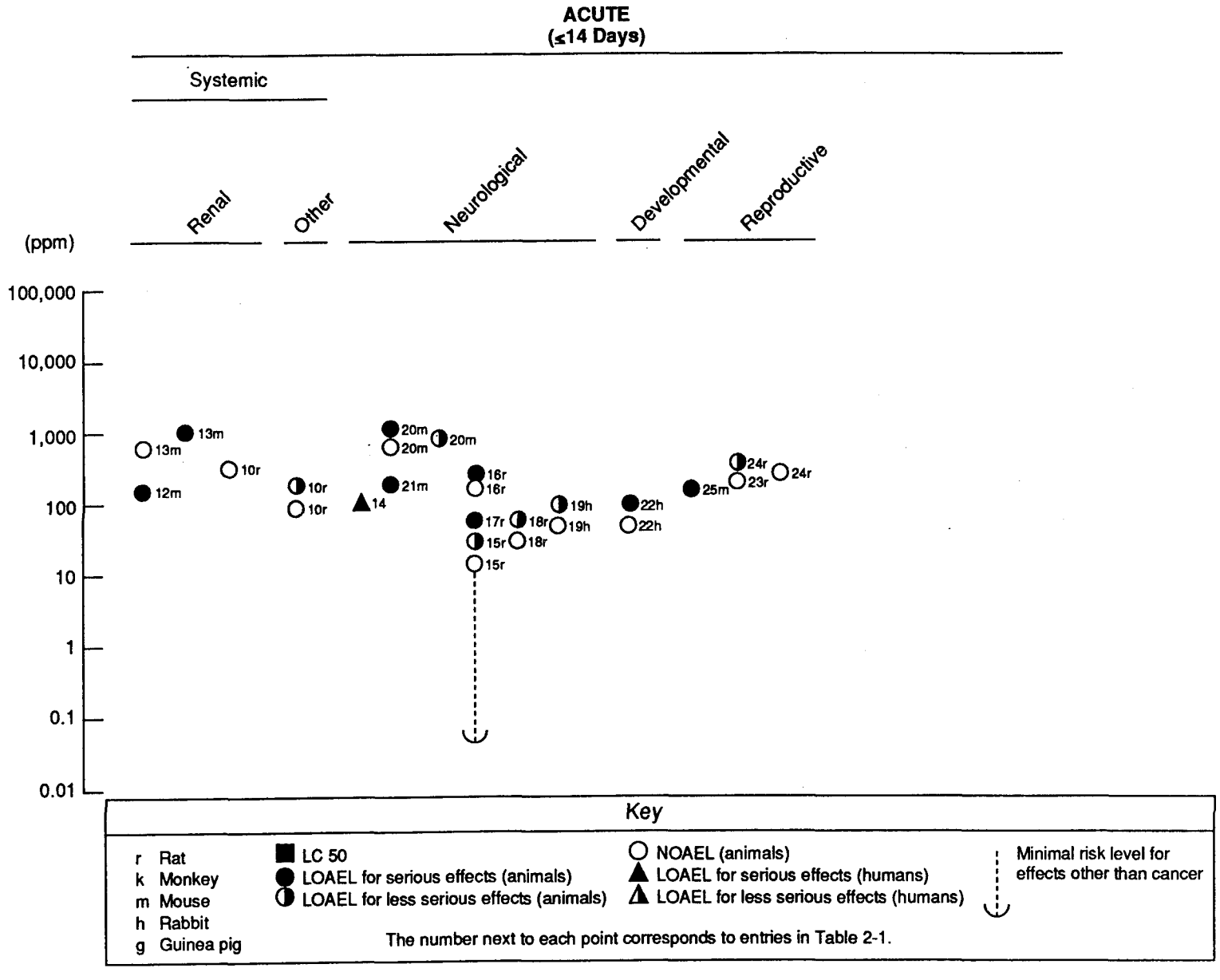


FIGURE 2-1 (Continued)

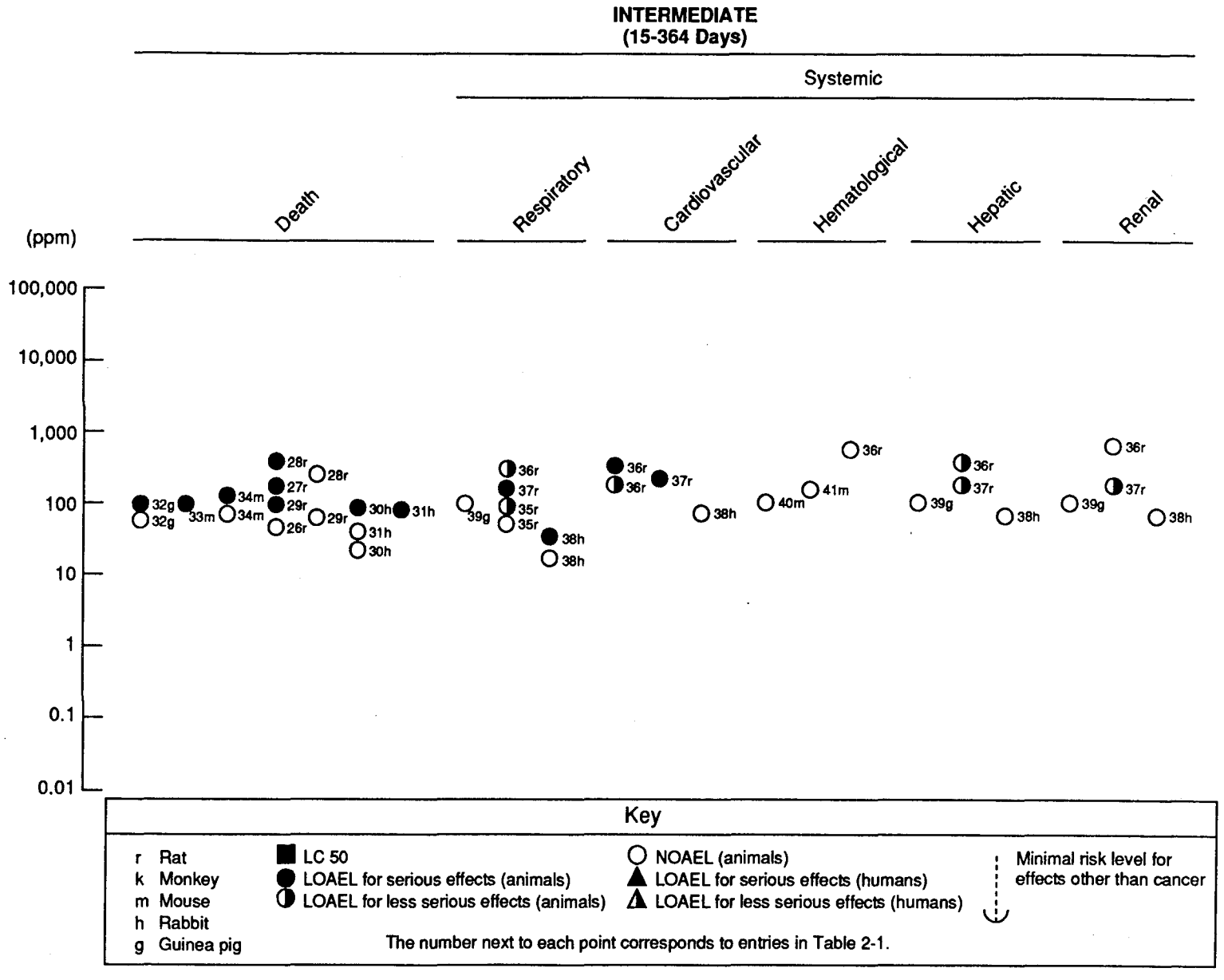


FIGURE 2-1 (Continued)

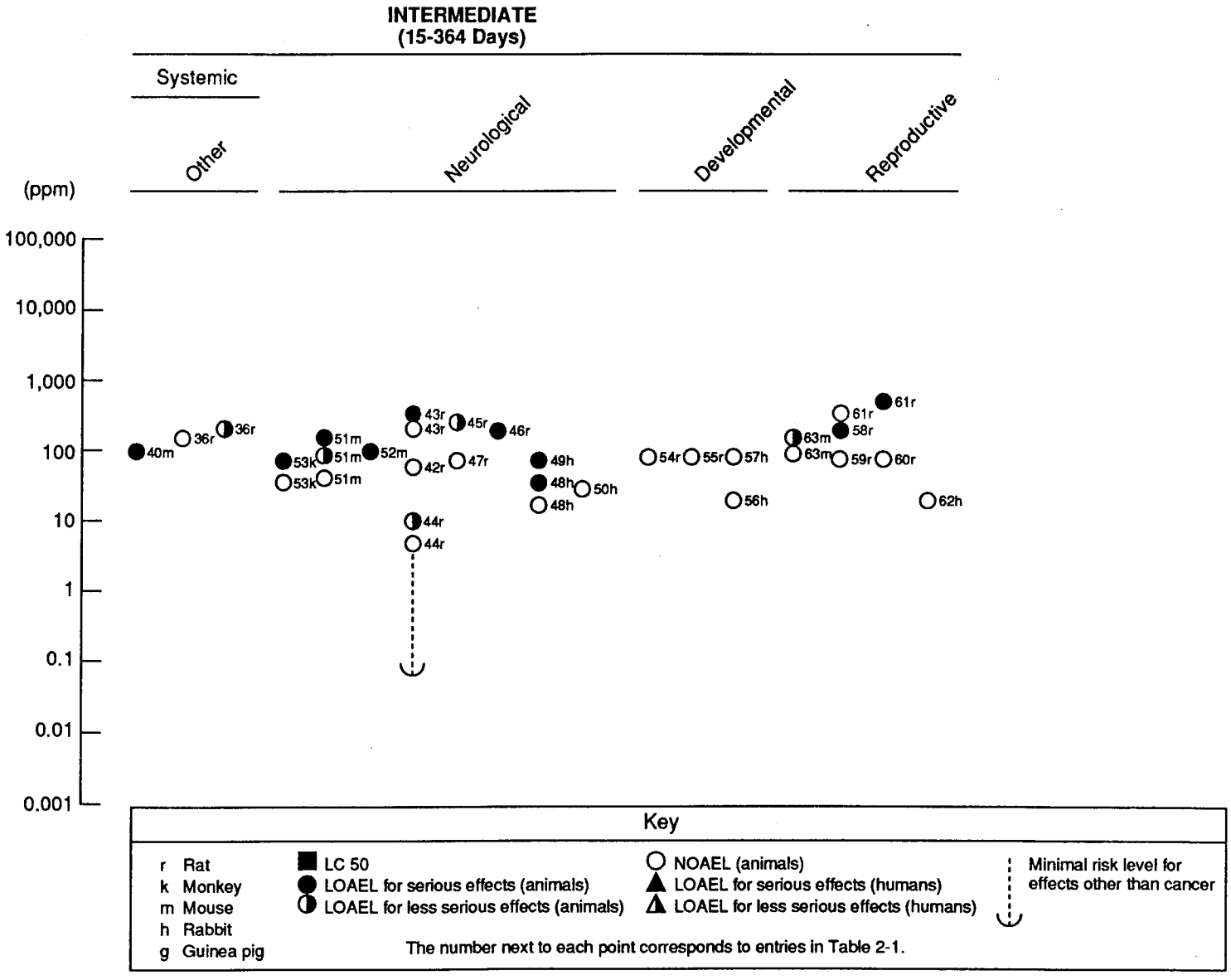
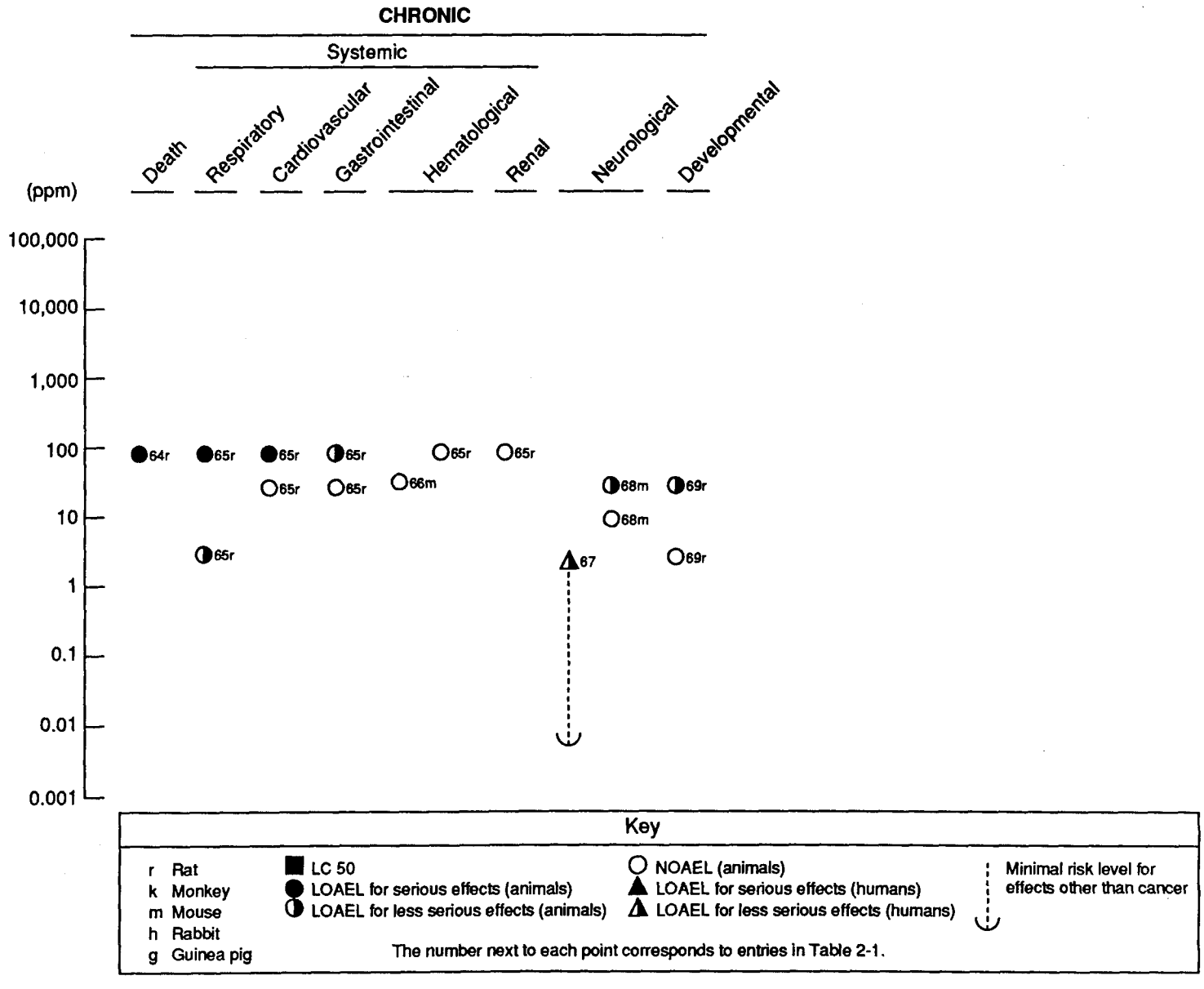


FIGURE 2-1 (Continued)



2.2.1.2 Systemic Effects

No studies were located regarding musculoskeletal effects on humans or animals after inhalation exposure to bromomethane. Information on other systemic effects is presented below. The highest NOAEL values and all reliable LOAEL values for systemic effects in each species and duration category are recorded in Table 2-l and plotted in Figure 2-l.

Respiratory Effects. Studies in humans indicate that the lung may be severely injured by inhalation exposure to bromomethane. Edema is the most common effect, and is often accompanied by focal hemorrhagic lesions (Greenberg 1971; Marraccini et al. 1983; Miller 1943; Prain and Smith 1952; Wyers 1945). This injury can severely impair respiratory function and lead to hypoxia, cyanosis, and complete respiratory failure (Greenberg 1971; Hine 1969; O'Neal 1987). Similar edematous and hemorrhagic lesions are seen in lungs of several rodent species exposed to bromomethane (Irish et al. 1940; Kato et al. 1986; Reuzel et al. 1987; Sato et al. 1985) and severe damage can also occur to the nasal epithelium (Eustis et al. 1988; Hurtt et al. 1987a, 1988a). As shown in Table 2-l and Figure 2-1, these effects occur mainly at acute exposure levels of 100 ppm or higher, but may occur at levels of 3-10 ppm if exposure duration is extended (Reuzel et al. 1987; Sato et al. 1985).

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after inhalation exposure to bromomethane, but several studies in mice and rats indicate that the heart is susceptible to injury. Effects which have been repor.ted at exposure levels of 90-160 ppm include cardiomyopathy (Eustis et al. 1988), myocardial degeneration and cardiac thrombi (Reuzel et al. 1987) and fibrosis (Kato et al. 1988).

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans after inhalation exposure to bromomethane. Gastrointestinal effects have not been noted in most animal studies, but Alexeeff et al. (1985) reported an unusual increase in hemorrhagic lesions of the colon in mice exposed to high concentrations (1,490 ppm) of bromomethane, and Reuzel et al. (1987) noted an increased incidence of hyperkeratosis of the esophagus and stomach in rats exposed to 90 ppm. This effect is probably mediated by transport of bromomethane from the lungs to the throat by mucociliary clearance (Reuzel et al. 1987).

Hematological Effects. Hematological effects have not been observed in humans exposed to bromomethane (Johnstone 1945; Kantarjian and Shasheen 1963; Longley and Jones 1965; O'Neal 1987; Viner 1945; Wyers 1945). In animals, decreased red and white blood cell counts were noted in one study of rats exposed to 160 ppm (Eustis et al. 1988), but hematological effects were not detected by others (Drew 1984; Haber 1987; Kato et al. 1986; Reuzel et al. 1987). These studies indicate that blood and blood-forming cells are not important target tissues for bromomethane.

Hepatic Effects. Case reports of humans exposed to bromomethane vapors indicate the liver may become swollen and tender in some cases (Hine 1969; O'Neal 1987; Miller 1943; Prain and Smith 1952), but often no significant liver injury is detected (Greenberg 1971; Hine 1969; Marraccini et al. 1983). Similar results have been reported in animals, with mild signs of liver injury (edema, focal hemorrhages, minimal necrosis) being noted in some studies at levels of 150-600 ppm (Alexeeff et al. 1985; Eustis et al. 1988; Hurtt et al. 1987a; Kato et al. 1986), with no significant injury at levels of 66 ppm (Irish et al. 1940).

Renal Effects. Adverse renal effects are often reported in humans exposed to high levels of bromomethane vapor. Common effects include congestion, anuria or oliguria, and proteinuria (Hine 1969; Marraccini et al. 1983; O'Neal 1987; Prain and Smith 1952; Viner 1945; Wyers 1945). However, there are many cases where renal effects are minimal or absent (Hine 1969; Johnstone 1945; Longley and Jones 1965). Similar signs of renal injury have been reported in several animal studies, including swelling, edema, nephrosis, and tubular necrosis (Alexeeff et al. 1985; Eustis et al. 1988).

Dermal/Ocular Effects. Bromomethane vapor is irritating to the skin and eyes, and humans who are exposed to bromomethane in air may experience conjunctivitis, erythema, rashes, or even blisters (Marraccini et al. 1983; O'Neal 1987; Wyers 1945). The effects of direct dermal or ocular contact with bromomethane vapors are discussed more fully in Section 2.2.3.2. No studies were located regarding dermal or ocular effects following systemic absorption of bromomethane in animals or humans.

2.2.1.3 Immunological Effects

No studies were located regarding immunological effects in humans or animals after inhalation exposure to bromomethane.

2.2.1.4 Neurological Effects

Inhalation exposure to bromomethane frequently leads to a spectrum of neurological effects in humans. Initial symptoms typically include headache, nausea, confusion, weakness, numbness, and visual disturbances (Anger et al. 1986; Hine 1969; Kantarjian and Shasheen 1963; Marraccini et al. 1983; O'Neal 1987; Rathus and Landy 1961; Watrous 1942). In severe cases, these effects may progress to ataxia, tremor, seizures, paralysis, and coma (Behrens and Dukes 1986; Greenberg 1971; Longley and Jones 1965; Marraccini et al, 1983; O'Neal 1987; Prain and Smith 1952; Prockop and Smith 1986; Rathus and Landy 1961; Viner 1945; Wyers 1945). In most cases of acute exposure, the effects do not occur immediately, but develop after a lag of several hours (Clarke et al. 1945). If death does not ensue, symptoms usually decrease in severity over the course of several weeks to several months, although frequently they do not disappear completely (Chavez et al. 1985; Greenberg 1971; Hine 1969; Johnstone 1945; Kantarjian and Shasheen 1963; Longley and Jones 1965; Prockop and Smith 1986).

Quantitative data on the exposure levels leading to neurological effects in humans are limited. Early studies indicated that workplace exposure to concentrations of 100 ppm or even less for 1-2 weeks could lead to headache, nausea, numbness, and ataxia (Johnstone 1945; Watrous 1942). Longer-term exposure (8 years) to average levels of 2.3 ppm are suspected to be the cause of an increased incidence of neurological symptoms (muscle ache, .fatigue, ataxia) in a group of fumigators who used bromomethane (Anger et al. 1986). Based on this value, a chronic inhalation MRL of 0.005 ppm was calculated, as described in footnote "d" of Table 2-1.

Inhalation studies in animals confirm that the central nervous system is injured by inhalation exposure to bromomethane. Clinical effects that have been detected include tremors, ataxia, paralysis, and seizures (Alexeeff et al. 1985; Anger et al. 1981; Breslin et al. 1990; Drew 1984; Haber 1987; Hurtt et al. 1987a; Irish et al. 1940; Kato et al. 1986). Histological lesions in the brain (focal necrosis and hemorrhage) have also been detected (Alexeeff et al. 1985; Eustis et al. 1988; Hurtt et al. 1987a). As shown in Table 2-l and Figure 2-1, neurological effects are typically observed at exposure levels ranging between 80 and 1,000 ppm, although longer-term (6 month to 2 year) exposure of rabbits, monkeys, or mice has led to functional neurological impairment at concentrations of 33-66 ppm (Haber 1987; Irish et al. 1940). As in humans, these effects tend to be at least partly reversible when exposure ceases (Irish et al. 1940).

The most sensitive indicator of neurological effects in animals appears to be alterations in the levels of neurotransmitters in the brain. In rats exposed to bromomethane for 8 hours, there was a significant decrease in the hypothalamic concentration of norepinephrine and a decrease in the activity of tyrosine hydroxylase at an exposure level of 31 ppm, but not at 16 ppm (Honma 1987). Based on this value (16 ppm), an acute inhalation MRL value of 0.05 ppm was calculated as described in footnote "b" of Table 2-1. When exposure duration was extended to 3 weeks (24 hours/day), dramatic decreases in norepinephrine and 5-hydroxytryptamine were detected at exposure levels of 10 ppm but not at 5 ppm (Honma et al. 1982). Based on this value (5 ppm), an intermediate inhalation MRL of 0.05 ppm was calculated as described in footnote "c" of Table 2-1.

2.2.1.5 Developmental Effects

No studies were located regarding developmental effects in humans after inhalation exposure to bromomethane. Several studies in rats and rabbits indicate that inhalation exposure to levels up to 70 ppm during gestation does not result in any significant developmental effects, even when there is severe maternal toxicity (Hardin et al. 1981; Sikov et al. 1980). However, an increased incidence of anomalies.and malformations was observed in offspring from rabbits exposed to 80 ppm during gestation (Breslin et al. 1990), and decreased pup weights were noted in a multigeneration study in rats exposed to 30 ppm (Enloe et al. 1986). The NOAEL and LOAEL values are recorded in Table 2-1 and plotted in Figure 2-l.

2.2.1.6 Reproductive Effects

No studies were located regarding reproductive effects in humans after inhalation exposure to bromomethane. In male animals, effects on the testes (delayed spermiation, tubular degeneration, atrophy) have been observed in rats and mice exposed to 160-405 ppm for 1-6 weeks (Eustis et al. 1988; Hurtt et al. 1987a; Kato et al. 1988) or 120 ppm for 13 weeks (Drew 1984). However, exposure of male rats to 70 ppm for 5 days did not interfere with normal reproductive function and impregnation success (McGregor 1981). No effects on* reproductive function in females have been observed in rats or rabbits exposed to levels up to 70 ppm before and during gestation (Sikov et al. 1980), even though these levels produce maternal toxicity. The highest NOAEL values and all reliable LOAEL values for reproductive effects in each species and duration category are recorded in Table 2-l and plotted in Figure 2-l.

2.2.1.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans after inhalation exposure to bromomethane. In animals, the frequency of bone marrow cells with chromosomal aberrations was not increased in rats exposed to 70 ppm for 5 days (McGregor 1981), but was increased several-fold in rats exposed to 140 ppm for 14 days (Ikawa et al. 1986). Djalali-Behzad et al. (1981) found that inhalation exposure of mice to bromomethane for 4 hours led to alkylation of DNA in liver and spleen, although the levels were quite low. In contrast to these positive findings, no genotoxic effects could be detected in sperm from rats or mice exposed to 70 ppm bromomethane for 5 days, using either the dominant lethal or recessive lethal tests, or by direct examination of the sperm (McGregor 1981). These studies indicate that bromomethane does have genotoxic potential, but that effects may be minimal and difficult to measure following brief or low dose exposure. Other genotoxicity studies are discussed in Section 2.4.

2.2.1.8 Cancer

The carcinogenic potential of bromomethane has not been formally investigated in epidemiological studies of occupationally-exposed workers. Wong et al. (1984) studied the incidence of cancer in a cohort of workers exposed to a wide variety of brominated chemicals, and noted that two men who died of testicular cancer had both been exposed to bromomethane. However, since there are numerous risk factors for testicular cancer, and since the workers may have been exposed to other chemicals, this observation is not sufficient to indicate that bromomethane is carcinogenic. No evidence of carcinogenic effects was detected in mice exposed to 33 ppm for 2 years (Yang 1990), or in rats exposed to 90 ppm for 29 months (Reuzel et al. 1987).

2.2.2 Oral Exposure

Because bromomethane is a gas under ordinary conditions, the oral toxicity of this compound has not been thoroughly studied. No information was located regarding health effects in humans after oral exposure to

bromomethane. Available data from oral studies in animals are summarized in Table 2-2 and Figure 2-2, and this information is discussed below.

2.2.2.1 Death

No studies were located regarding lethality in humans or animals after oral exposure to bromomethane.

2.2.2.2 Systemic Effects

No studies were located regarding cardiovascular, musculoskeletal, renal, or dermal/ocular effects in humans or animals after oral exposure to bromomethane. Information on other systemic effects is presented below. The highest NOAEL values and all reliable LOAEL values for these systemic effects are recorded in Table 2-2 and plotted in Figure 2-2.

Respiratory Effects. No studies were located regarding respiratory effects in humans after oral exposure to bromomethane. In animals, no histological evidence of lung injury was detected in rats exposed to oral doses of 50 mg/kg/day for 13 weeks (Danse et al. 1984). Slight atelectasis was observed in some animals exposed to oral doses of 10 or 50 mg/kg/day, but this was judged to be due to inadvertent inhalation exposure that occurred during oral dosing (Danse et al. 1984).

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans after oral exposure to bromomethane. Several studies in animals have shown that repeated (90-day) administration of concentrated solutions of bromomethane (40-5,000 mg/L, dissolved in oil) by gavage to rats can result in irritation and hyperplasia of the epithelium in the forestomach (Boorman et al. 1986; Danse et al. 1984). No effects were observed in animals administered 0.4 mg/kg/day (Danse et al. 1984). Mild focal hyperemia was detected at concentrations of 200 mg/L (equivalent to a dose of 2 mg/kg/day), with hyperplasia, hyperkeratosis, and fibrosis developing after repeated administration of 1,000 mg/L (equivalent to a dose of 10 mg/kg/day) or higher. Repeated doses of 5,000 mg/L (50 mg/kg/day) caused frank ulcerations of the forestomach. These lesions appear to be the result of a direct irritant effect of bromomethane on the epithelium. The epithelial hyperplasia regresses when exposure is stopped, although fibrotic lesions or adhesions which developed during exposure remain (Boorman et al. 1986). The possible relationship between this hyperplastic response and cancer of the forestomach is discussed below in Section 2.2.2.8.

Based on the data of Danse et al. (1984), it is judged that doses up to 0.4 mg/kg/day do not produce significant adverse effects on the stomach, and this dose has been used to derive an intermediate oral MRL of 0.003 mg/kg/day as described in footnote "b" of Table 2-2.

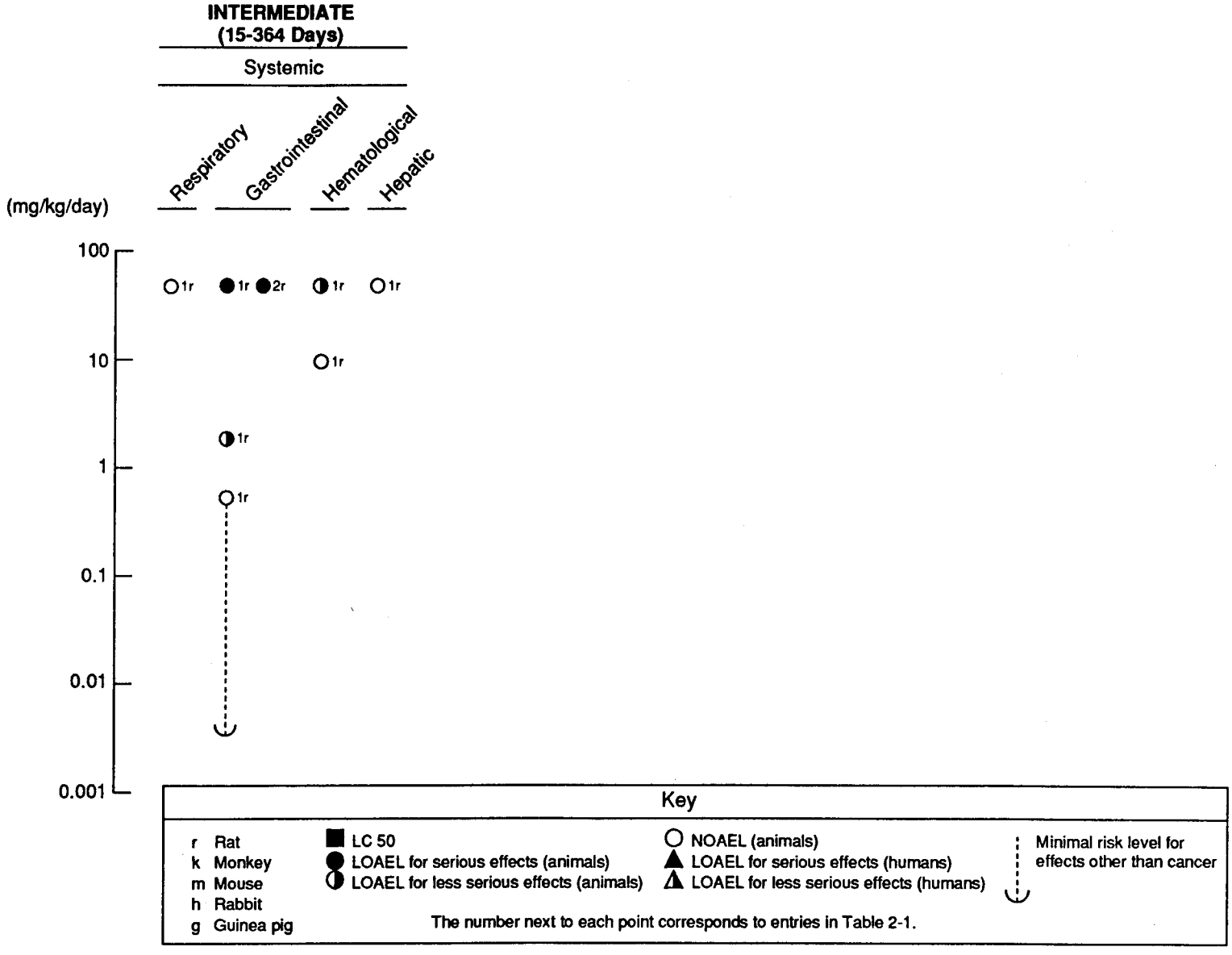
TABLE 2-2. Levels of Significant Exposure to Bromomethane - Oral

Key to figure[a]	Species	Route	Exposure frequency/ duration	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
INTERMEDIATE EXPOSURE								
Systemic								
1	Rat	(G)	13 wk 5d/wk	Resp	50			Danse et al. 1984
				Gastro	0.4[b]	2 (hyperplasia, focal hyperemia)	50 (ulcers)	
				Hemato	10	50 (slight anemia)		
				Hepatic	50			
2	Rat	(G)	13-25 wk 5d/wk	Gastro			50 (fibrosis, inflammation, hyperplasia)	Boorman et al. 1986

[a]The number corresponds to entries in Figure 2-2.
[b]Used to derive an intermediate duration oral Minimal Risk Level (MRL) of 0.003 mg/kg/day; dose adjusted for intermittent exposure (5d/wk) and divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans, and 10 for human variability).

d = day(s); (G) = gavage - not specified; Gastro = gastrointestinal; Hemato = hematological; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s)

FIGURE 2-2. Levels of Significant Exposure to Bromomethane – Oral



Hematological Effects. No studies were located regarding hematological effects in humans after oral exposure to bromomethane. Slight anemia was observed in rats exposed to doses of 50 mg/kg/day for 13 weeks, but this was judged to be secondary to the pronounced lesions of the forestomach (Danse et al. 1984). No evidence of other hematological effects was detected at doses up to 10 mg/kg/day.

Hepatic Effects. No studies were located regarding hepatic effects in humans after oral exposure to bromomethane. In animals, histological signs of liver damage were not detected in rats given doses up to 50 mg/kg/day for 90 days (Danse et al. 1984).

Other Systemic Effects. Rats exposed to oral doses of 50 mg/kg/day for 90 days did not gain weight normally (Boorman et al. 1986; Danse et al. 1984). This effect was probably secondary to decreased food intake, and was not observed at doses up to 10 mg/kg/day (Danse et al. 1984).

2.2.2.3 Immunological Effects

No studies were located regarding immunological effects in humans or animals after oral exposure to bromomethane.

2.2.2.4 Neurological Effects

No studies were located regarding neurological effects in humans or animals after oral exposure to bromomethane.

2.2.2.5 Developmental Effects

No studies were located regarding developmental effects in humans or animals after oral exposure to bromomethane.

2.2.2.6 Reproductive Effects

No studies were located regarding reproductive effects in humans or animals after oral exposure to bromomethane.

2.2.2.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.4.

2.2.2.8 Cancer

No studies were located regarding carcinogenic effects in humans after oral exposure to bromomethane. Danse et al. (1984) exposed rats by gavage to bromomethane (in oil) for 90 days, and observed a dose-dependent increase in the incidence of forestomach lesions which were interpreted as squamous cell carcinomas. However, histological diagnosis of epithelial carcinomas in the presence of marked hyperplasia is difficult (Wester and Kroes 1988). After reevaluation of the histological slides, a panel of scientists from the

National Toxicology Program (NTP) concluded that there was no evidence of a neoplastic response in this study, but rather only hyperplasia and inflammation (IRIS 1989). This is consistent with the observation that the hyperplasia of the forestomach produced by 13 weeks of exposure to bromomethane regresses when exposure is ended (Boorman et al. 1986). However, longer-term (25 week) oral exposure of rats to 50 mg/kg/day of bromomethane led to a forestomach lesion in one rat (out of 15 exposed) that was judged to be a very early carcinoma. Although this observation is not adequate to draw firm conclusions, these data suggest that the initial hyperplasia produced by bromomethane may occasionally lead to neoplasia after sufficient time.

2.2.3 Dermal Exposure

The effects of dermal contact with bromomethane have been described in numerous case reports of humans who were exposed either to liquid bromomethane (mainly from fire extinguishers) or bromomethane vapors (mainly during fumigation activities). These studies are discussed below. No studies were located regarding dermal exposure of animals to bromomethane.

2.2.3.1 Death

No cases were located in which dermal exposure to bromomethane led to death in humans.

2.2.3.2 Systemic Effects

Adverse effects on the respiratory system, cardiovascular system, gastrointestinal tract, blood, musculoskeletal system, liver, or kidneys have not been observed in humans exposed to bromomethane by the dermal route (e.g., Butler et al. 1945; Hine 1969; Wyers 1945; Zwaveling et al. 1987).

Dermal/Ocular Effects. Direct dermal contact with bromomethane can lead to severe injury to the skin. Symptoms usually do not appear immediately, but develop a few hours after exposure. Early signs typically include a burning or itching sensation, with erythema, edema, and large blisters that resemble second-degree burns developing somewhat later (Butler et al. 1945; Hezemans-Boer et al. 1988; Watrous 1942; Wyers 1945). Injury is usually mild on exposed skin areas where rapid evaporation can occur and is more severe in moist or covered regions where evaporation is retarded (Watrous 1942; Zwaveling et al. 1987). Effects generally begin to subside within 5-10 days after exposure (Watrous 1942), and recovery is usually complete within about 1 month (Butler et al. 1945; Zwaveling et al. 1987).

The exposure levels leading to dermal effects of this sort are rarely known. Most cases involve people doused with liquid bromomethane (Longley and Jones 1965; Watrous 1942) or exposed to very high vapor levels (Hezemans-Boer et al. 1988; Zwaveling et al. 1987) (see Table 2-3). Numerous case reports of humans exposed to lower levels of bromomethane fumes did not include

TABLE 2-3. Levels of Significant Exposure to Bromomethane Vapor - Dermal

Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
ACUTE EXPOSURE						
Systemic						
Human	40 min (1 exp)	Derm/oc			10000 (blisters)	Hezemans-Boer et al. 1988

Derm/oc = dermal/ocular; exp = exposure; LOAEL = lowest-observed-adverse-effect level; min = minute(s); NOAEL = no-observed-adverse-effect level

descriptions of dermal effects, even though the level of inhalation exposure caused profound or even fatal neurological or respiratory effects (e.g., Greenberg 1971; Hine 1989; Marraccini et al. 1983).

No studies were located regarding the following effects in humans or animals after dermal exposure to bromomethane:

2.2.3.3 Immunological Effects
2.2.3.4 Neurological Effects
2.2.3.5 Developmental Effects
2.2.3.6 Reproductive Effects

2.2.3.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.4.

2.2.3.8 Cancer

No studies were located regarding cancer in humans or animals after dermal exposure to bromomethane.

2.3 TOXICOKINETICS

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

No studies were located regarding the amount of bromomethane absorbed by humans during inhalation exposure. Several studies in rats indicate that the rate of bromomethane uptake across the lung is directly proportional to the concentration in air, 1.6 $(kg\ hr)^{-1}$ with estimated rate constants ranging from 0.32 to (Gargas and Andersen 1982; Medinsky et al. 1985). Fractional absorption appears to be about 50% at exposure levels up to around 180 ppm (Medinsky et al. 1985). At high levels (310 ppm), the total amount absorbed appears to reach a maximum (62 mg/kg), suggesting that some aspect of uptake (perhaps glutathione availability) becomes limiting (see Section 2.3.3).

2.3.1.2 Oral Exposure

No studies were located regarding bromomethane absorption after oral exposure of humans. In rats given a single oral dose of ^{14}C-labeled bromomethane dissolved in corn oil, only about 3% of the label was excreted in the feces (Medinsky et al. 1984). This indicates that at least 97% of the dose was absorbed from the gastrointestinal tract.

2.3.1.3 Dermal Exposure

No quantitative studies were located regarding bromomethane absorption across the skin of humans or animals.

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

No studies were located regarding bromomethane distribution in humans after inhalation exposure. In rats exposed to ^{14}C-bromomethane in air, radioactive label was widely distributed throughout the body (Bond et al. 1985; Jaskot et al. 1988; Medinsky et al. 1985). Levels were somewhat higher in lung, adrenal, liver, and kidney than in other tissues (Bond et al. 1985; Jaskot et al. 1988). The form of the label was not studied by these researchers, but is probably mostly metabolites. However, Honma et al. (1985) showed that low levels of parent bromomethane can be detected for up to 24 hours after exposure.

2.3.2.2 Oral Exposure

No studies were located regarding bromomethane distribution in humans after oral exposure. In rats given oral doses of ^{14}C-bromomethane, label was distributed widely throughout the body, with highest levels in liver and kidney (Medinsky et al. 1984).

2.3.2.3 Dermal Exposure

No studies were located on bromomethane distribution in humans or animals after dermal exposure.

2.3.3 Metabolism

Bromomethane undergoes initial metabolism primarily by nucleophilic displacement of the bromide ion. When the attacking species is water, the products are methanol and bromide ion:

$$HOH + CH_3Br \rightarrow CH_3OH + H^+ + Br^-$$

The amount of bromomethane broken down by this reaction in the body is not known, but increased levels of both methanol and bromide have been detected in exposed animals (Gargas and Andersen 1982; Honma et al. 1985). Bromomethane may also react with organic thiols (R-SH) to yield S-methyl derivatives:

$$R\text{-}SH + CH^3Br \rightarrow R\text{-}SCH^3 + H^+ + Br^-$$

This has been shown to result in formation of S-methylcysteine derivatives in hemoglobin of mice exposed to bromomethane (Iwasaki 1988b), and by analogy with methyl chloride (Kornburst and Bus 1983), is likely to result in formation of S-methyl glutathione (Medinsky et al. 1985). Further metabolism of methanol or S-methyl derivatives such as those mentioned above then leads to the formation of carbon dioxide (generally accounting for 40%-50% of the administered dose) and other unidentified nonvolatile metabolites (generally accounting for about 20%-25% of the dose) (Bond et al. 1985; Jaskot et al. 1988; Medinsky et al. 1985).

2.3.4 Excretion

2.3.4.1 Inhalation Exposure

No studies were located regarding excretion of bromomethane in humans after inhalation exposure. In animals exposed to bromomethane vapors, excretion occurs mainly by expiration of carbon dioxide or by urinary excretion of nonvolatile metabolites (Bond et al. 1985; Jaskot et al. 1988; Medinsky et al. 1985). Only small amounts are excreted in the feces. Very little parent bromomethane is exhaled (Jaskot et al. 1988; Medinsky et al. 1985), and tissue levels of parent bromomethane decrease with a half-life of only about 15-30 minutes (Honma et al. 1985; Jaskot et al. 1988). Half-lives for clearance of metabolites from the body and most tissues range from 2 to 10 hours (Honma et al. 1985; Jaskot et al. 1988).

A significant fraction (about 25%-30%) of ^{14}C-label remains in tissues after 24-72 hours and is excreted more slowly (Jaskot et al. 1988; Medinsky et al. 1985). This slow excretion of label presumably represents turnover of various intracellular metabolites or adducts, although this has not been established.

2.3.4.2 Oral Exposure

No studies were located regarding excretion of bromomethane by humans after oral exposure. One study in animals indicates that the rate and pattern of excretion of ^{14}C-label following oral exposure to ^{14}C-bromomethane is similar to that following inhalation exposure: 32% was exhaled as carbon dioxide, 43% was excreted in the urine, 4% of unmetabolized parent compound was exhaled, 2% was excreted in the feces, and 14% remained in the body after 72 hours (Medinsky et al. 1984).

2.3.4.3 Dermal Exposure

No studies were located regarding excretion of bromomethane by humans or animals after dermal exposure.

2.4 RELEVANCE TO PUBLIC HEALTH

Bromomethane exists as a gas at ordinary temperatures, so the most likely route of human exposure is by inhalation. The hazard of this compound is increased by the fact that it has very little odor at potentially toxic levels (Alexeeff and Kilgore 1983), and effects on the body are generally delayed. Thus, people may be exposed to hazardous levels without being aware that the exposure is occurring.

There are a number of studies that provide good quantitative doseresponse data by the inhalation route, and inhalation MRLs have been derived for acute, intermediate and chronic inhalation exposure. The acute value is based on a study in rats in which exposure to 31 ppm for 8 hours caused altered levels of brain neurotransmitters, while 16 ppm had no effect (Honma

1987). The MRL of 0.05 ppm was obtained by adjusting the NOAEL (16 ppm) for less than continuous exposure (8 hour/day) and dividing by an uncertainty factor of 100 (10 for extrapolation from animals to humans, and 10 for human variability). The intermediate-duration inhalation MRL is based on a 3-week study in rats in which exposure to 10 ppm resulted in decreased brain neurotransmitters, while 5 ppm did not (Honma et al. 1982). The intermediate MRL of 0.05 ppm was derived from the NOAEL (5 ppm) by dividing by an uncertainty factor of 100, as described above. The chronic inhalation MRL is based on an epidemiological study of workers who had an increased prevalence of muscle ache, fatigue, and ataxia following chronic exposure to average levels of 2.3 ppm (Anger et al. 1986). The MRL was derived by adjusting this LOAEL (2.3 ppm) to account for noncontinuous exposure (8 hr/day, 5 days/week), and by dividing by an uncertainty factor of 100 (10 for use of a LOAEL, and 10 for human variability).

Because bromomethane tends to volatilize and exists mainly as a gas at room temperature, only two oral toxicity studies have been performed (Boorman et al. 1986; Danse et al. 1984). Both studies were performed by administering bromomethane dissolved in oil to rats and both studies reported that irritation of the stomach was the chief effect. The 13-week study by Danse et al. (1984) identified a NOAEL of 0.4 mg/kg/day and a LOAEL of 2 mg/kg/day. An intermediate-duration oral MRL of 0.003 mg/kg/day was derived from the NOAEL (0.4 mg/kg/day) by adjusting for intermittent exposure (5 days/week) and dividing by an uncertainty factor of 100 (10 for extrapolation from animals to humans, and 10 for human variability).

No dermal MRLs have been derived for bromomethane due to the lack of an appropriate methodology for development of dermal MRLs.

More detailed information on the adverse effects associated with exposure to bromomethane is presented below.

Death. Many people have died following accidental inhalation exposure to high levels of bromomethane (Alexeeff and Kilgore 1983). The exposure levels leading to death in humans are not precisely defined, with estimates ranging from 1,600 to 60,000 ppm, depending on duration of exposure (Holling and Clarke 1944; Miller 1943; Wyers 1945). Lethal exposure levels in animals also depend on duration of exposure, with mortality occurring in rats and rabbits after 24 hours exposure to 220 ppm (Irish et al. 1940), or after 2 weeks to 2 years exposure to 66-100 ppm (Drew 1984; Haber 1987; Hardin et al. 1981; Ikeda et al. 1980; Irish et al. 1940; Reuzel et al. 1987). Exposure to a lethal concentration of bromomethane is only likely to occur in the immediate vicinity of fumigation activities or a major spill, and is not of concern under normal circumstances.

Systemic Effects.

Respiratory Effects. Acute inhalation exposure of humans and animals to bromomethane can result in marked lung irritation (edema, hemorrhagic lesions), and this may lead to moderate to severe impairment of respiratory

function (Greenberg 1971; O'Neal 1987; Prain and Smith 1952). Dose-response data are limited for humans, but the effect range in animals is usually lo-1,000 ppm (see Figure 2-l). Exposures to concentrations of bromomethane this high are not likely near waste sites unless a major spill or accident occurs. Oral exposure does not appear to result in lung injury (Danse et al. 1984).

Renal Effects. The kidney also is sensitive to bromomethane. Anuria and proteinuria are common signs of renal injury in acutely exposed humans (O'Neal 1987; Prain and Smith 1952; Viner 1945), but dose-response data are not available. In animals, nephrosis has been noted in rats and mice exposed to 160 ppm for 2-6 weeks (Eustis et al. 1988).

Hepatic Effects. Mild effects (congestion, focal hemorrhages) are sometimes observed in liver and other tissues (O'Neal 1987; Prain and Smith 1952; Wyers 1945), but these effects do not appear to be as significant as the respiratory and renal injury.

Gastrointestinal Effects. Although data are limited, the only systemic tissue that has been found to be affected following oral exposure is the epithelium of the stomach (Boorman et al. 1986; Danse et al. 1984). This is presumably a result of direct contact between bromomethane and the gastrointestinal epi,thelium. Based on this effect, an intermediate-duration oral MRL of 0.003 mg/kg/day has been derived. It is likely that this effect (which has only been observed in animals dosed with concentrated solutions of bromomethane dissolved in oil) would be much less pronounced if exposure occurred via ingestion of more dilute solutions of bromomethane in water. However, this has not been studied.

Dermal/Ocular Effects. Direct dermal contact with bromomethane can cause mild to severe skin lesions such as erythema, itching, and blisters (Butler et al. 1945; Hezemans-Boer et al. 1988; Watrous 1942). These lesions, which normally heal within 2-4 weeks after exposure, have only been noted after exposure to liquid bromomethane or very high concentrations of vapor, and are unlikely to occur in persons exposed to bromomethane in the environment.

Immunological Effects. No studies were located regarding immunological effects in humans or animals after exposure to bromomethane. In the absence of any data, it is not possible to predict whether this is an effect of concern in exposed humans.

Neurological Effects. Humans acutely exposed to high concentrations of bromomethane vapor nearly always experience injury to the central nervous system. Initial effects, which usually occur within a few hours of exposure, include headache, weakness, and nausea (Marraccini et al. 1983; Wyers 1945), and may also include blurred or double vision (Chavez et al. 1985; Johnstone 1945). Depending on exposure level, these symptoms may progress into ataxia, tremors, and clonic seizures (Prain and Smith 1952; Prockop and Smith 1986). These effects typically begin to wane after several days, but recovery may not

be complete even after many months (Longley and Jones 1965; Rathus and Landy 1961). In rats, brain neurotransmitter levels were decreased following an acute exposure to 31 ppm (Honma 1987). Based on the NOAEL of 16 ppm identified by this study, an acute inhalation MRL of 0.05 ppm has been derived.

Only limited information is available on the effects of long-term inhalation exposure of humans to low levels of bromomethane. Headache, weakness, and increased prevalence of neurological signs such as muscle ache, fatigue, dizziness, and ataxia have been noted in workers exposed for extended periods in the workplace (Anger et al. 1986; Hine 1989; Kantarjian and Shasheen 1963; Kishi et al. 1988). No cases of severe neurological effects from long-term exposure to low levels have been noted in humans, but intermediate or chronic inhalation exposure of animals to bromomethane vapor is known to result in moderate to severe neurological injury. Rabbits and monkeys appear to be the most sensitive species, with convulsions and paralysis occurring at exposure levels of 33-66 ppm for 6 months (Irish et al. 1940). Continuous exposure to concentrations of 10 ppm decreased neurotransmitter levels in rats (Honma et al. 1982). Based on the NOAEL of 5 ppm identified by this study, an intermediate inhalation MRL of 0.05 ppm has been derived. The chronic exposure level leading to neurological effects in humans is not known precisely, but Anger et al. (1986) reported mild effects in workers exposed to an average of about 2.3 ppm. Based on this, a chronic inhalation MRL of 0.005 ppm has been derived.

No studies were located regarding neurological effects in humans or animals following oral exposure to bromomethane. Based on the clear neurological effects produced by inhalation exposure, it seems likely such effects would also be of concern following acute or repeated oral intake of adequate doses.

The mechanism of bromomethane-induced neurotoxicity is not known. It is generally agreed that effects are not the result of metabolic breakdown products such as methanol or bromide, since neither the characteristic effects nor the dose dependency correspond to those of the metabolites (Clarke et al. 1945; Honma et al. 1985). Rather, it is more likely that bromomethane acts by alkylating key cellular components such as enzymes (Lewis 1948; Rathus and Landy 1961).

Developmental Effects. No studies were located regarding developmental effects in humans after exposure to bromomethane. Several studies of animals exposed to bromomethane vapors up to 70 ppm did not detect developmental effects, even though these concentration levels resulted in maternal toxicity (Hardin et al. 1981; Sikov et al. 1980). However, exposure of rabbits to 80 ppm during gestation resulted in increased incidence of several developmental anomalies in the off-spring (Breslin et al. 1990). These data suggest bromomethane may cause developmental effects, but only at high doses where other effects would also be of concern.

Reproductive Effects. No studies were located regarding reproductive effects in humans after exposure to bromomethane. Inhalation exposure of male rats to high levels of bromomethane (120-400 ppm) has resulted in decreased sperm production along with testicular degeneration and atrophy (Drew 1984; Eustis et al. 1988; Kato et al. 1986). However, exposures up to 70 ppm do not appear to interfere with reproductive functions in male (McGregor 1981) or female rats (Hardin et al. 1981; McGregor 1981; Sikov et al. 1980). It is difficult to judge from these data whether adverse reproductive effects are likely to occur in exposed humans, but it seems probable that the respiratory, neurological, and renal effects will normally be of greatest clinical concern.

Genotoxic Effects. Bromomethane has produced positive results in a number of mutagenicity test systems, both in vitro (Table 2-4) and in vivo (Table 2-5). This effect does not appear to require metabolic activation, which is consistent with the fact the bromomethane is a direct-acting alkylating agent which can methylate DNA (Ikawa et al. 1986; Starratt and Bond 1988). This property suggests that bromomethane might be carcinogenic, but this has not been established (see below).

Carcinogenic Effects. No epidemiological studies were located on cancer incidence in humans exposed specifically to bromomethane. One study of workers exposed to a variety of brominated chemicals noted that bromomethane was the only common exposure of two men who died of testicular cancer (Wong et al. 1984). However, this does not establish that bromomethane was the causative agent. Chronic inhalation studies performed in mice and rats revealed no evidence of carcinogenic effects at exposure levels of 33-90 ppm (Reuzel et al. 1987; Yang 1990). Rats given daily oral doses of 50 mg/kg/day for 90 days developed inflammation and keratosis of the forestomach, along with lesions that were onginally interpreted as squamous carcinomas (Danse et al. 1984). However, reevaluation of the histological specimens by NTP scientists indicated that the forestomach lesions in this study were hyperplastic but not neoplastic. A subsequent study also found hyperplasia but no neoplasia in rat forestomach (Boorman et al. 1986) after 13 weeks of exposure. After 25 weeks, one animal developed a lesion that was judged to be a very early carcinoma. These results are too limited (both in number of animals and exposure duration) to draw firm conclusions, but in view of the alkylating ability and positive mutagenicity finding for this chemical, it seems possible that longer-term exposure might lead to measurable increases in tumor frequency, EPA considers that the data currently available are inadequate to evaluate the carcinogenic potential of bromomethane, and has assigned this chemical to Group D (not classifiable as to human carcinogenicity) (IRIS 1989).

Similarly, the International Agency for Research on Cancer has placed bromomethane in Group 3 (not classifiable as to carcinogenic potential).

TABLE 2-4. Genotoxicity of Bromomethane *In Vitro*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
Prokaryotic organisms:				
Escherichia coli Sd-4 (forward mutation)	Gene mutation	No data	+	Djalali-Behzad et al. 1981
E. Coli WP2 hcr (gene reversion)	Gene mutation	+	+	Moriya et al. 1983
Salmonella typhimurium (TA100, TA1535) (gene reversion)	Gene mutation	+	+	Moriya et al. 1983
S. typhimurium (TA98, TA1537, TA1538) (gene reversion)	Gene mutation	-	-	Moriya et al. 1983
S. typhimurium (TA100) (dessicator system)	Gene mutation	No data	-	Simmon and Tardiff 1978
S. typhimurium (TA98) (plate test)	Gene mutation	-	-	Kramers et al. 1985
S. typhimurium (TA100) (plate test)	Gene mutation	+	+	Kramers et al. 1985
Klebsiella pneumoniae (ur^- pro^-) (fluctuation test)	Gene mutation	No data	+	Kramers et al. 1985
Eukaryotic organisms:				
Mouse lymphoma cells (L5178YTK+/-) (forward mutation)	Gene mutation	No data	+	Kramers et al. 1985
Syrian hamster embryo cells	Enhanced transformation by SA7 adenovires	No data	-	Hatch et al. 1983
Human peripheral lymphocytes	Sister chromatid exchanges	No data	+	Tucker et al. 1986
Rat liver cells	Unscheduled DNA synthesis	No data	-	Kramers et al. 1985
Human embryonic intestinal cells	Unscheduled DNA synthesis	-	-	McGregor 1981

+ = positive result; - = negative result; (+) = weakly positive result

TABLE 2-5. Genotoxicity of Bromomethane *In Vivo*

Species (test system)	End point	Results	Reference
Nonmammalian systems:			
Drosophila melanogaster Berlin-K wild type (sex-linked recessive lethal test)	Gene mutation	+	Kramers et al. 1985
D. melanogaster (somatic wing spot assay)	Recombinogenic activity	+	Katz 1987
D. melanogaster Oregon-K wild type (sex-linked recessive lethal test)	Gene mutation	-	McGregor 1981
Mammialian systems:			
Rat (bone marrow cells) CD Sprague-Dawley	Chromosome aberrations	-	McGregor 1981
Rat CD Sprague-Dawley	Dominant lethal	-	McGregor 1981
Mouse B6C3F1	Sperm abnormality	-	McGregor 1981
Mouse (liver and spleen cells) CBA	DNA alklyation	+	Djalali-Behzad et al. 1981
Rat (bone marrow cells) F344	Micronuclei inductions	+	Ikawa et al. 1986
Mouse (bone marrow cells) BDF_1	Micronuclei inductions	+	Ikawa et al. 1986

+ = positive result; - = negative result

2.5 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time biologic samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to bromomethane are discussed in Section 2.5.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are often not substance specific. They also may not be directly adverse, but can'indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by bromomethane are discussed in Section 2.5.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, biologically effective dose, or target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.7, "POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE."

2.5.1 Biomarkers Used to Identify and/or Quantify Exposure to Bromomethane

The most convenient biomarker of bromomethane exposure is the concentration of bromide ion in the blood or serum. The relationship between bromide ion concentrations and the severity of effects in exposed people was

investigated by Alexeeff and Kilgore (1983), who assembled and evaluated data from a large number of case reports. Serum bromide levels are usually below 15 ppm in unexposed people. In bromomethane-exposed people, levels up to 80 ppm may occur without any obvious clinical signs, while levels of 150-400 ppm are observed in people with moderate to severe symptoms. Bromide is cleared from blood with a half-life of about 12 days in healthy people, and half-lives of 3-15 days have been observed in bromomethane-exposed people (Alexeeff and Kilgore 1983). Consequently, the correlation between serum bromide levels and severity of effects is most apparent within the first l-2 days of exposure, and there may be little correlation later. Bromide ion is cleared mainly by excretion in the urine, but no studies were located on the use of urinary bromide levels as a biomarker of bromomethane exposure.

Measurement of parent bromomethane (e.g., in expired air, blood, or urine) has not been investigated as a possible biomarker of exposure in humans, mainly because studies in animals suggest that bromomethane is cleared so rapidly (half-lives of 15-30 min) that this is unlikely to be useful for monitoring environmental exposures. Similarly, methanol and other organic metabolites are also cleared with short half-lives (Honma et al. 1985; Jaskot et al. 1988), so they are also unlikely to be useful in biomonitoring. Formation of stable methylated adducts such as S-methylcysteine in hemoglobin is known to occur in animals following inhalation exposure to bromomethane (Iwasaki 1988a, 1988b), but this has not been developed as a biomonitoring method for humans.

Neither elevated serum bromide levels nor formation of methylated adducts are, by themselves, specific for bromomethane exposure. For example, increased bromide levels could result from exposure to bromide in the diet or ingestion of bromate- or bromide-containing medicines, and increased methyl adducts might result from exposure to other methyl halides, various methyl nitrosoamines, or other alkylating agents. However, the combination of these two methods (i.e., a finding of increased bromide and increased methylation) would strongly indicate that bromomethane exposure had occurred.

2.5.2 Biomarkers Used to Characterize Effects Caused by Bromomethane

As discussed in Section 2.2, the effects that are most often observed in humans exposed to bromomethane vapor are central nervous system injury (disturbed vision, tremor, convulsions, coma), lung irritation (edema, impaired respiration), and renal injury (oliguria or anuria). Of these, neurological or neurobehavioral signs appear to be the most sensitive indication of effect, since preclinical symptoms can be observed in humans exposed to low levels of bromomethane in the workplace (Anger et al. 1986; Kishi et al. 1988; Verberk et al. 1979). Of course, positive findings for end points of this sort (headache, weakness, ataxia, nausea, double vision, abnormal electroencephalogram) are not specific indicators of bromomethane exposure, since other chemicals or diseases may produce similar neurological changes. Biomarkers that are useful in evaluating neurological effects have been discussed by Johnson (1987).

2.6 INTERACTIONS WITH OTHER CHEMICALS

No studies were located regarding the interaction of bromomethane with other chemicals. Since it seems likely that cellular glutathione may serve a protective function by reacting with bromomethane (Kornburst and Bus 1983), other chemicals (electrophilic xenobiotics, reactive intermediates) that lead to decreases in glutathione levels might increase the toxicity of bromomethane, but this has not been investigated. Similarly, bromomethane might be expected to have additive or synergistic interactions with other alkylating agents, but this has not been investigated.

2.7 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

No studies were located to suggest that any specific human subpopulation may be more susceptible to bromomethane than average, although it may be expected that the young, the elderly, and people with lung, kidney, or neurological disease might be more readily affected than healthy adults. Studies in animals reveal that there are differences in sensitivity between species (e.g., Irish et al. 1940), and some studies have noted small differences in sensitivity between males and females (Eustis et al. 1988). It is not known if these differences apply to humans.

2.8 MITIGATION OF EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to bromomethane. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to bromomethane. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

Human exposure to bromomethane is most likely to occur by inhalation or dermal contact (see Chapter 5). Inhalation exposure may cause neurological, respiratory and renal damage. Dermal contact may cause skin lesions while oral exposure leads to digestive tract mucosal membrane irritation (see Section 2.2).

Procedures that have been used to reduce absorption of bromomethane include the following. If dermal exposure to concentrated bromomethane occurs, contaminated clothing is removed and the skin thoroughly washed with soap or mild detergent and water (Bradford 1990; Ellenhorn and Barceloux 1988; Morgan 1982). General burn care may also be necessary in severe cases. If inhalation exposure is sufficient to cause lung damage, administration of oxygen, mechanical ventilatory support, and administration of diuretics and bronchodilators may be required to reduce the effects of pulmonary edema (Bradford 1990; Morgan 1982). If seizures occur, treatment with standard anticonvulsants may be required. It is unlikely that exposures near waste sites would be large enough to require interventions of this sort.

Intramuscular administration of sulfhydryl agents such as dimercaprol has been recommended to improve elimination of bromomethane, since bromomethane reacts with sulfhydryl groups (Bradford 1990). However, this treatment may cause troublesome side effects, and there is no evidence that such agents are effective when administered after exposure has occurred (Alexeeff and Kilgore 1983; Ellenhorn and Barceloux 1988; Rathus and Landy 1961). Treatment with N-acetylcysteine has been suggested, since this compound is a precursor to glutathione, and elevated glutathione levels may be protective against bromomethane toxicity (Bradford 1990). It is not expected that treatments of this sort would normally be required for low dose exposures that occur near waste sites. Additional details regarding treatment following bromomethane intoxication may be found in the cited references.

2.9 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of bromomethane is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of bromomethane.

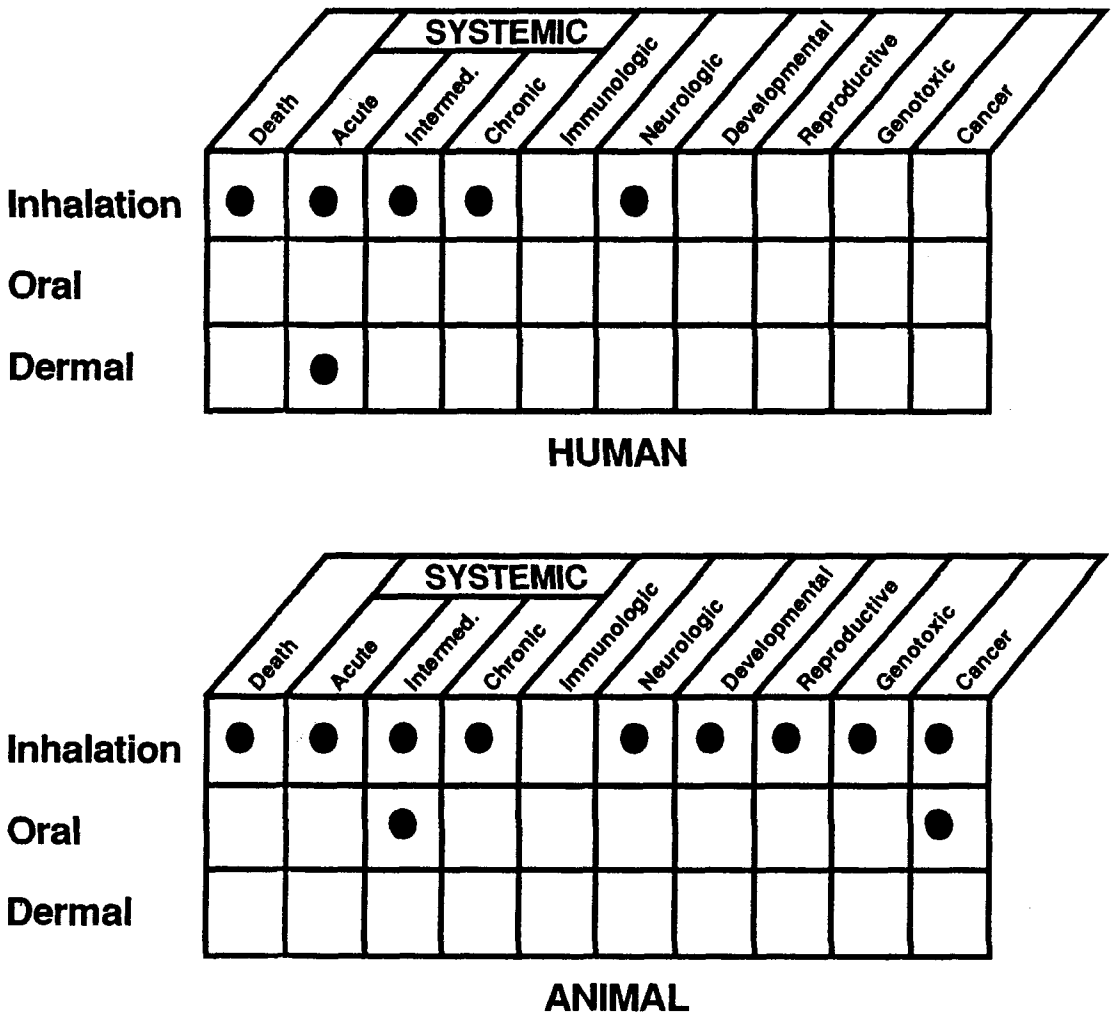
The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that, if met, would reduce or eliminate the uncertainties of human health assessment. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

2.9.1 Existing Information on Health Effects of Bromomethane

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to bromomethane are summarized in Figure 2-3. The purpose of this figure is to illustrate the existing information concerning the health effects of bromomethane. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not imply anything about the quality of the study or studies. Gaps in this figure should not be interpreted as "data needs" information (i.e., data gaps that must necessarily be filled).

As shown in the upper portion of Figure 2-3, there are numerous studies of humans exposed to bromomethane by the inhalation route. These have focused mainly on the systemic and neurological effects of exposure, and other endpoints (immunological, developmental, reproductive, genotoxic, cancer) have not been investigated. There are also numerous case reports on the direct effects of bromomethane vapor or liquid on skin, but other effects have not been studied.

FIGURE 2-3. Existing Information on Health Effects of Bromomethane



● Existing Studies

Studies in animals (shown in the lower half of Figure 2-3) have also focused on inhalation exposure, and most endpoints (except immunotoxicity) have been investigated. In contrast, the effects of oral exposure have received only limited attention, focusing mainly on the inflammatory and possible carcinogenic effects in the stomach. No information was located on dermal exposure of animals.

2.9.2 Data Needs

Acute-Duration Exposure. There is sufficient information from studies of humans (Holling and Clarke 1944; Johnstone 1945; Miller 1943; Wyers 1945) to identify the principal target tissues of bromomethane following acute inhalation exposure (lung, kidney, nervous system). Studies in animals (Alexeeff et al. 1985; Eustis et al. 1988; Honma 1987; Honma et al. 1985, 1987; Hurtt et al. 1987a, 1988a; Hurtt and Working 1988) support and confirm these observations. These data are sufficient to derive an acute inhalation MRL (Honma 1987). Information is available on dermal effects following exposure of humans (but not animals) both to the vapor (Hezemans-Boer et al. 1988; Zwaveling et al. 1987) and the liquid (Longley and Jones 1965; Watrous 1942). While the dose-response curve for dermal effects is not well-defined, it is apparent that this is of concern mainly at high levels, and is unlikely to be of concern at exposure levels likely to be encountered in the environment or near waste sites.

No information is available on acute oral exposure of humans or animals to bromomethane. Extrapolation from acute inhalation data is probably not appropriate, since some of the effects (both inhalation and oral) are due to point-of-contact irritation. However, acute oral toxicity studies are probably not essential, since oral exposure of humans to acutely toxic levels of bromomethane is not likely to occur due to the high volatility of the compound.

Intermediate-Duration Exposure. Limited information is available on the effects of intermediate-duration inhalation exposure of humans to bromomethane (Kantarjian and Shasheen 1963; Viner 1945). It appears clear that the target tissues are the same as for acute-duration exposure, but dose-response data from intermediate-duration human studies are not available. However, there are a number of studies in animals that do provide quantitative data (Anger et al. 1981; Eustis et al. 1988; Haber 1987; Honma et al. 1982; Ikeda et al. 1980; Irish et al. 1940; Kato et al. 1986), and are sufficient to derive an intermediate-duration inhalation MRL (Honma et al. 1982). Further data from an intermediate-duration (90-day) study in animals recently completed by the NTP will help strengthen this data set, and additional intermediate-duration inhalation studies may not be required.

No information is available on the effects of intermediate-duration oral exposure in humans, but two animal studies (Boorman et al. 1986; Danse et al. 1984) provide sufficient data to identify the main target tissue (the stomach epithelium) and to define the dose-response relationship for this effect. These studies are suitable for derivation of an intermediate oral MKL, but

further studies would still be helpful to search more specifically for possible subclinical neurological effects. This is important since neurological effects appear to be the most sensitive effect by the inhalation route, and people may be exposed to low levels of bromomethane in drinking water drawn from contaminated groundwater sources. No information is available on intermediate-duration dermal exposure to bromomethane. However, humans are not likely to experience significant dermal exposures to bromomethane near waste sites, so research in this area does not appear to be essential.

Chronic-Duration Exposure and Cancer. There are several studies of humans chronically exposed to bromomethane in air (Anger et al. 1986; Chavez et al. 1985; Hine 1969; Kishi et al. 1988). These studies indicate neurological effects are the most sensitive effect following chronic exposure. Quantitative exposure data are limited, but are sufficient for derivation of a chronic inhalation MRL. The human data are supported by chronic inhalation studies in animals (Anger et al. 1986; Haber 1987). Nevertheless, further studies of humans exposed to low levels of bromomethane in the workplace would be helpful in order to increase the confidence in the chronic MRL. This is important since humans could be exposed to low levels of bromomethane in air near some waste sites. No information is available on effects in humans or animals after chronic oral exposure. Extrapolation from the inhalation route may not be appropriate, since two intermediate-duration studies in animals (Boorman et al. 1986; Danse et al. 1984) indicate that the stomach and not the nervous system is the main target following oral exposure. Chronic oral studies in animals would be helpful in evaluating human health risk by this route. This is important since humans might be exposed to low levels of bromomethane in drinking water drawn from contaminated groundwater sources.

No information is available from studies of humans on the carcinogenic effects of inhalation exposure to bromomethane, but chronic inhalation studies in mice and rats (Reuzel et al. 1987; Yang 1990)yielded no evidence of carcinogenic effect. Nevertheless, epidemiological investigations of the incidence of cancer in workers who use bromomethane would be helpful in assessing the cancer risk to humans who could be exposed to low levels of bromomethane in air near some waste sites.

The carcinogenic effects of oral exposure to bromomethane have been studied in two intermediate-duration studies (Boorman et al. 1986; Danse et al. 1984), but not in any chronic studies. The results suggest that bromomethane might be carcinogenic, but the data are difficult to interpret with certainty. Extrapolation from inhalation studies would not be appropriate, since the response observed is at the portal of entry (the forestomach). This is consistent with the concept that bromomethane is a direct-acting alkylating agent. Chronic oral exposure studies in animals would be valuable for clarifying the cancer risk of ingested bromomethane. This is needed since some people could be exposed to low levels of bromomethane in drinking water drawn from contaminated groundwater sources.

Genotoxicity. Bromomethane is a direct-acting alkylating agent, and there are studies both in vivo (Djalali-Behzad et al. 1981) and in vitro (Starratt and Bond 1988) which establish that it can methylate DNA. Studies of mutagenic potential in bacterial test systems have been mostly positive (Djalali-Behzad et al. 1981; Kramers et al. 1985; Moriya et al. 1983), as have several in vitro tests using eukaryotic cell types (Kramers et al. 1985; Tucker et al. 1986) and several in vivo tests in animals (Ikawa et al. 1986; Katz 1987; Kramers et al. 1985). Investigation of possible sister chromatid exchange or chromosome aberrations in peripheral lymphocytes of humans exposed in the workplace would be helpful in confirming the genotoxic potential of bromomethane, although studies in animals suggest that this effect may only be measurable at high exposure levels.

Reproductive Toxicity. No information was located regarding reproductive effects in humans. Intermediate-duration inhalation studies in animals (Eustis et al. 1988; Kato et al. 1986) indicate that the testes may undergo degeneration and atrophy at high exposure levels, but the doseresponse curve is not well defined. Further studies in animals to identify the threshold for this end point would be helpful in confirming that neurological effects are the most sensitive endpoint of toxicity. Two studies in female animals (Hardin et al. 1981; Sikov et al. 1980) have not detected reproductive effects even at doses that produced maternal toxicity. Additional studies to confirm this in several different animal species would be helpful.

No information exists on reproductive effects in humans or animals after oral exposure. Based on the inhalation studies in animals which indicate the testes are a target tissue, it would be valuable to include histological examination of the testes in any intermediate- or chronic-duration oral studies in animals. In addition, tests of male reproductive success would be valuable in assessing the functional significance of any testicular lesions.

Developmental Toxicity. There is no information on developmental effects in humans exposed to bromomethane, but two inhalation exposure studies in animals (rats and rabbits) indicate that developmental or teratogenic effects do not occur even at doses that are toxic to the dam (Hardin et al. 1981; Sikov et al. 1980). No information is available on developmental effects after oral exposure of animals to bromomethane, but the inhalation data suggest that is not likely to be of concern.

Immunotoxicity. No information was located on the immunological effects of bromomethane in humans or animals exposed by any route. A battery of immune function tests in several animal species exposed to bromomethane by the inhalation and the oral routes would be valuable in determining if the immune system is adversely affected, and if so, in determining species and route specificity, as well as the threshold for those effects.

Neurotoxicity. There is clear evidence from studies in humans and animals that the nervous system is adversely affected by inhalation exposure to bromomethane. This includes evidence of clinical neurological signs and

behavioral changes (Anger et al. 1986; Behrens and Dukes 1986; Clarke et al. 1945; Greenberg 1971; Hine 1969; Kantarjian and Shasheen 1963; Longley and Jones 1965; Marraccini et al. 1983; O'Neal 1987; Prain and Smith 1952; Prockop and Smith 1986; Rathus and Landy 1961; Viner 1945; Wyers 1945), as well as biochemical changes and histological lesions in the brain (Alexeeff et al. 1985; Eustis et al. 1988; Honma 1987; Honma et al. 1982; Hurtt et al. 1987a). Although quantitative exposure information from humans is limited, the thresholds for acute, intermediate, and chronic inhalation exposures are known with reasonable precision. No information is available on humans exposed by the oral route, but two oral studies in rats (Boorman et al. 1986; Danse et al. 1984) did not produce any visible neurological signs. It is not known if this apparent route specificity is due simply to differences in dose, or to differences in absorption, distribution, or metabolism between routes. For this reason, additional oral dose-response studies in animals that focus specifically on histological, biochemical, or functional tests of nervous system injury would be valuable. If these tests indicate that the nervous system is not injured following oral exposure, additional toxicokinetic studies would be helpful in understanding the basis for the distinction between inhalation and oral effects.

Epidemiological and Human Dosimetry Studies. As noted previously, there are many reports on the adverse effects of bromomethane in humans. Most studies involve people with accidental acute high-level exposures in air, but there are also several studies of workers with repeated low-level exposures (Anger et al. 1986; Kishi et al. 1988; Verberk et al. 1979). These studies are sufficient to identify the main health effects of concern and to estimate the exposure levels that lead to effects. However, further studies of workers who are exposed to low levels during manufacture or use of bromomethane would be helpful, if reliable current and past exposure data are available. These additional quantitative human data would be valuable in increasing the confidence in the estimated safe exposure levels in the workplace and the environment. This would improve the ability to evaluate potential risk to humans exposed to low levels of bromomethane in air near waste sites.

Biomarkers of Exposure and Effect. The most common biomarker of exposure to bromomethane is serum bromide concentration. Studies in humans have established a correlation between bromide levels and severity of effect (Alexeeff and Kilgore 1983), although the quantitative relation between exposure level and bromide concentration has not been established. Since bromide is cleared from the blood with a half-life of 3-15 days, this test is best suited for detecting relatively recent exposures. Because bromide is a normal component of blood, and because bromide levels may be increased by other chemicals or drugs, increased serum bromide is not specific for bromomethane. Other possible biomarkers available include direct measurement of parent bromomethane or methanol in expired air or blood (Honma et al. 1985; Jaskot et al. 1988), and measurement of methylated adducts such as S-methylcysteine in hemoglobin (Iwasaki 1988a). Measurement of parent bromomethane or methanol are not likely to be helpful except in the interval immediately following an acute exposure, while measurement of stable methyl adducts could be useful for longer periods. Further studies in humans or

animals would be helpful in determining the sensitivity of these biomarkers and evaluating their usefulness in monitoring people exposed to low levels of bromomethane near waste sites.

The most sensitive biomarkers of bromomethane effect appear to be changes in the nervous system. These can be detected in groups of exposed people by measuring the incidence of signs and symptoms such as weakness, nausea, ataxia, and vision problems. However, it is obvious that these are not specific for bromomethane-induced effects, and because of the large variation between people, these tests are not reliable for identifying preclinical effects in potentially exposed individuals. Studies to develop more specific and more objective biomarkers of bromomethane-induced effects would be useful in assessing the potential health significance of low-level bromomethane exposure near waste sites.

Absorption, Distribution, Metabolism, and Excretion. The toxicokinetics of bromomethane have not been thoroughly investigated in humans, but there is good information from studies in animals on uptake, distribution, and excretion following inhalation exposure (Bond et al. 1985; Gargas and Andersen 1982; Honma et al. 1985; Jaskot et al. 1988; Medinsky et al. 1985), and there is one study on toxicokinetics following oral exposure (Medinsky et al. 1984). Available data indicate that the toxicokinetics of bromomethane absorption are mainly first-order except at very high doses. While the metabolism of related compounds such as chloromethane has been studied in detail (Kornburst and Bus 1983), the metabolism of bromomethane has not been thoroughly investigated. Additional studies on the rate and extent of bromomethane hydrolysis and alkylation reactions in vivo would be valuable in understanding the basis of bromomethane toxicity, and in assessing the utility of various biomarkers of exposure (e.g., parent compound, bromide, methanol, adducts).

Comparative Toxicokinetics. Available studies indicate that bromomethane affects the same target tissues in humans and animals, although there are apparent differences in sensitivity between species, with rabbits being more sensitive than rats or mice (Irish et al. 1940). However, quantitative toxicokinetic data on absorption, distribution, and excretion are available only for rats (Bond et al. 1985; Gargas and Andersen 1982; Honma et al. 1985; Jaskot et al. 1988; Medinsky **et** al. 1984, 1985). Additional toxicokinetic studies would be helpful in understanding the basis of the differences in species sensitivity, and in determining which animal species is the most appropriate model for human exposure.

Mitigation of Effects. Recommended methods for the mitigation of acute effects of inhalation exposure to bromomethane include mechanical ventilatory support, administration of oxygen and supportive therapy for pulmonary edema (Bradford 1990; Morgan 1982). Administration of thiol compounds to react with absorbed bromomethane has also been suggested (Bradford 1990). Further studies on the efficacy of post-exposure treatment with agents of this type would be valuable. No information was located concerning mitigation of effects of lower-level or longer-term exposure to bromomethane. Further information on techniques to mitigate such effects would be useful in

determining the safety and effectiveness of possible methods for treating bromomethane-exposed populations surrounding hazardous waste sites.

2.9.3 On-going Studies

The NTP has recently completed a series of inhalation studies in rats and mice, including both noncancer and cancer evaluations. The results of these studies will provide valuable new data on the toxicity of this compound in animals. Dr. W. Kilgore (University of California, Davis) is studying methods for detecting exposure of field workers to bromomethane, and is obtaining data on health effects in these workers.

3. CHEMICAL AND PHYSICAL INFORMATION

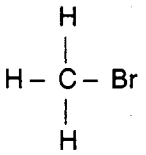
3.1 CHEMICAL IDENTITY

Table 3-l lists common synonyms, trade names, and other pertinent identification information for bromomethane.

3.2 PHYSICAL AND CHEMICAL PROPERTIES

Table 3-2 lists important physical and chemical properties of bromomethane.

TABLE 3-1. Chemical Identity of Bromomethane

Characteristic	Information	Reference
Chemical name	Bromomethane	Windholz 1983
Synonyms	Methyl bromide; monobromomethane; methyl fume	IRIS 1989
Trade names	Embafume®; Terabol®	EPA 1986b
Chemical formula	CH_3Br	Windholz 1983
Chemical structure		Windholz 1983
Identification numbers:		
CAS registry	74-83-9	Sax and Lewis 1987
NIOSH RTECS	PA-900000	HSDB 1989
EPA hazardous waste	U029	NLM 1989
OHM/TADS	No data	
DOT/UN/NA/IMCO shipping	UN1062	NLM 1989
	IMCO 2.3	HSDB 1989
	NA1581	HSDB 1989
HSDB	799	NLM 1989
NCI	No data	

CAS = Chemical Abstracts Service; DOT/UN/NA/IMCO = Department of Transportation/United Nations/North America/International Maritime Dangerous Goods Code; EPA = Environmental Protection Agency; HSDB = Hazardous Substances Data Bank; NCI = National Cancer Institute; NIOSH = National Institute for Occupational Safety and Health; OHM/TADS = Oil and Hazardous Materials/Technical Assistance Data System; RTECS = Registry of Toxic Effects of Chemical Substances

TABLE 3-2. Physical and Chemical Properties of Bromomethane

Property	Information	Reference
Molecular weight	94.95	Windholz 1983
Color	Colorless	Sax and Lewis 1987
Physical state	Gas	Windholz 1983
Melting point	-93.7°C	Windholz 1983
Boiling point	3.6°C	Windholz 1983
Density at 20°C[a]	3.97	Windholz 1983
Odor	Chloroform-like	Windholz 1983
Odor threshold:		
Water	No data	
Air	80 mg/m^3 (20 ppm)	Ruth 1986
Solubility:		
Water at 20°C	0.9 g/L	Verschueren 1983
	13.4-18.1 g/L	EPA 1986b
	13 g/L	Lyman et al. 1982
Organic solvents	Freely soluble	Windholz 1983
Partition coefficients:		
Log K_{ow}	1.1	Callahan et al. 1979
Log K_{oc}	0.77	Mabey et al. 1982
Vapor pressure at 20°C	1,420 mmHg	Mabey et al. 1982
Henry's law constant:		
(20°C)	0.013 $atm \cdot m^3/mole$	Lyman et al. 1982
	0.197 $atm \cdot m^3/mole$	Mabey et al. 1982
Autoignition temperature	Nonflammable	EPA 1986b
Flashpoint	Nonflammable	EPA 1986b
Flammability limits	Nonflammable	EPA 1986b
Conversion factors	1 ppm = 3.95 mg/m^3	Verschueren 1983
	1 mg/m^3 = 0.25 ppm	Verschueren 1983
Explosive limits	Nonflammable	EPA 1986b

[a]Density of vapor relative to air.

4. PRODUCTION, IMPORT, USE, AND DISPOSAL

4.1 PRODUCTION

Bromomethane is produced by reaction of methanol with hydrobromic acid, followed by distillation of the product (HSDB 1989; IARC 1986; Windholz 1983). Table 4-1 summarizes information on U.S. companies that reported the manufacture or use of bromomethane in 1987 (TRI 1989). The quality of the TRI data must be viewed with caution since the 1987 data represent first-time, incomplete reporting by these facilities. Not all facilities that should have reported have done so. Of the companies that did report, only two facilities produced bromomethane for sale and distribution: the Ethyl Corporation production facility in Magnolia, Arkansas, and the Great Lakes Chemical Corporation production facility in El Dorado, Arkansas (HSDB 1989; SRI 1987, 1988, 1989; TRI 1989). The current combined production volume of these two facilities is approximately 19,500 metric tons (43 million pounds) (HSDB 1989; IARC 1986). This is nearly a two-fold increase over the production volume of 11,200 metric tons (25 million pounds) reported for 1972 (IARC 1986).

4.2 IMPORT/EXPORT

Imports of bromomethane were 735 metric tons (1.6 million pounds) in 1982, while exports were 2,130 metric tons (4.7 million pounds) in 1984 and 4,135 metric tons (9 million pounds) in 1987 (HSDB 1989). More detailed data regarding the import and export of bromomethane were not located.

4.3 USE

The primary use of bromomethane is as a soil or space fumigant for the control of insects, fungi, and rodents (EPA 1986b; HSDB 1989; IARC 1986). Space fumigation is usually performed by enclosing the structure in a sealed tent and releasing bromomethane gas inside, while soil fumigation is usually done by injecting bromomethane into the soil underneath a nonporous covering. Bromomethane is also used as a methylating agent in various chemical reactions, and as a solvent to extract oils from nuts, seeds, and wool. Bromomethane was also used in fire extinguishers in Europe from the 1920s through the 1940s (IARC 1986), but never gained widespread use as a fire extinguishing agent in the United States (Alexeeff and Kilgore 1983).

4.4 DISPOSAL

Because bromomethane is a gas above 3.6°C (38°F), most disposal is by release to the atmosphere (see Section 5.2.1). Disposal of liquid or solid wastes that contain bromomethane is regulated by federal restrictions which apply to hazardous substances (see Chapter 7).

TABLE 4-1. Facilities That Manufacture or Process Bromomethane[a]

Facility	Location	Maximum Amount on site (lbs)	Use
Amoco Chemical Company	Decatur, AL	100-999	As an impurity
Great Lakes Chemical Co. El Dorado-Main Plant	El Dorado, AR	1,000,000-9,999,999	For sale/distribution
Gerber Products Company	Fort Smith, AR	1,000-9,999	In ancillary or other uses
Ethyl Corporation	Magnolia, AR	1,000,000-9,999,999	Produce; for sale/distribution; as a byproduct
	Hollister, CA	100,000-999,999	As a formulation component
Asgrow Florida Company	Belle Glade, FL	No Data	As a formulation component
Hms Chemicals Inc	Palmetto, FL	100,000-999,999	As a formulation component
Florida Fertilizer Co. Inc.	Wauchula, FL	100,000-999,999	For sale/distribution; in repackaging
Hercules-Brunswick Plant	Brunswick, GA	1,000-9,999	In ancillary or other uses
Borden, Inc. Grocery & Specialty Products	Lowell, MA	10,000-99,999	In ancillary or other uses
Borden, Inc. Gorcery & Specialty Prds.	Warren, MI	1,000-9,999	In ancillary or other uses
Mobay Corporation - Agricultural Chemicals Div.	Kansas City, MO	100,000-999,999	As a reactant
Comet Delta, Inc.	Greenville, MS	1,000-9,999	In ancillary or other uses
Coastal Chemical Corporation	Greenville, NC	100,000-999,999	In re-packaging
The Pillsbury Company	Buffalo, NY	No Data	In ancillary or other uses
Hershey Chocolate U.s.a. Hershey Plant	Hershey, PA	1,000-9,999	In ancillary or other uses
Consolidated Cigar Corp.	Cayey, PR	No Data	In ancillary or other uses
Amoco Chemical Company Cooper River	Wando, SC	100-999	Produce; as a byproduct
Cargill Flour Milling	Saginaw, TX	1,000-9,999	In ancillary or other uses

[a]Derived from TRI 1989.

5. POTENTIAL FOR HUMAN EXPOSURE

5.1 OVERVIEW

Because bromomethane is a gas under ordinary conditions, humans are most likely to be exposed to bromomethane in air. Low levels can be detected in air around the globe, perhaps onginating from natural sources in the ocean. Somewhat higher levels occur in urban environments, due to release from industrial point sources and from use of leaded gasoline. Extremely high levels may be encountered in air where bromomethane is being used for fumigation. Trace levels have been detected in some groundwater samples, but levels in surface water and food are usually negligible. Bromomethane may also be generated in drinking water as the result of chlorination, but this has not yet been quantified.

Bromomethane in air is quite stable, undergoing breakdown by reaction with hydroxyl radicals with a half-life of about 11 months. Bromomethane in other media (water, soil) volatilizes sufficiently rapidly that breakdown in these media (via hydrolysis or reaction with organic components) is usually minor.

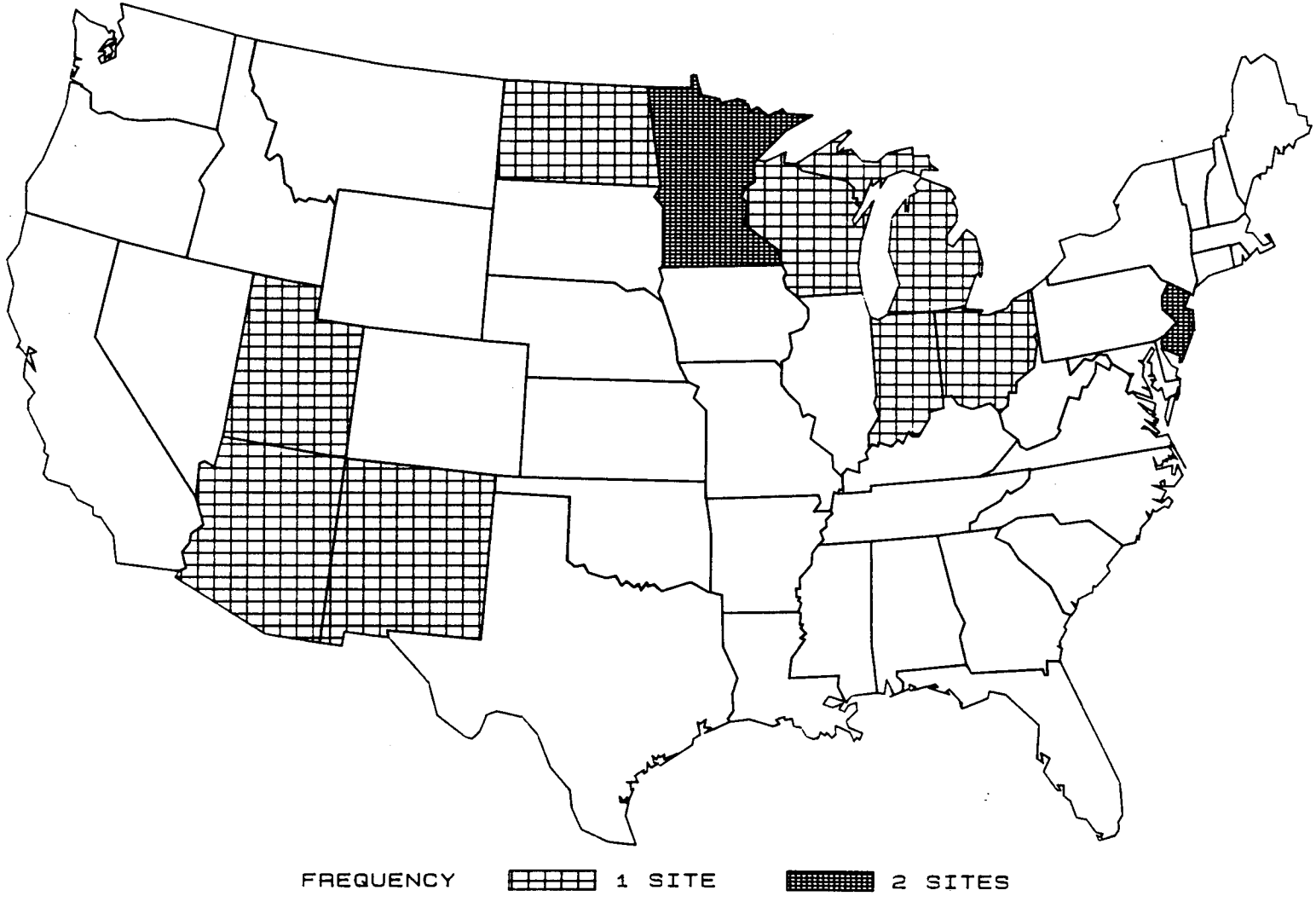
The EPA has identified 1,177 NPL sites. Bromomethane has been found at 12 of the sites evaluated for the presence of this chemical (View 1989). However, we do not know how many of the 1,177 sites have been evaluated for bromomethane. As more sites are evaluated by the EPA, the number may change. The frequency of sites where bromomethane has been found within the United States can be seen in Figure 5-1.

5.2 RELEASES TO THE ENVIRONMENT

5.2.1 Air

Since bromomethane is highly volatile, nearly all environmental releases of bromomethane are into the-air. The most important anthropogenic releases are from fumigation activities, since bromomethane is simply dispersed into the air after fumigation is completed. Based on current estimates that about 80% of bromomethane production is used for fumigation (65% for soil fumigation and 15% for space fumigation), and assuming that nearly all of this is ultimately released to air, approximately 34 million pounds/year may be released to air by this practice (HSDB 1989; IARC 1986). Air releases may also occur in association with industrial production and processing of bromomethane, as shown in Table 5-l (TRI 1989). Based on the data reported, total releases to air from industrial activities in the United States were 1.3 million pounds in 1987. However, the quality of the TRI data must be viewed with caution since the 1987 data represent first-time, incomplete reporting of estimated releases by these facilities. Not all sources of chemical wastes are included, and not all facilities that should have reported have done so.

FIGURE 5-1. FREQUENCY OF NPL SITES WITH BROMOMETHANE CONTAMINATION *



* Derived from View 1989

TABLE 5-1. Releases to the Environment from Facilities That Manufacture or Process Bromomethane[a]

Facility	Location	Total (lbs)						
		Air	Underground injection	Water	Land	Environment	POTW[b] transfer	Off-site transfer
Amoco Chemical Company	Decatur, AL	131,000	0	0	0	131,000	0	0
Great Lakes Chemical Co. El Dorado-Main Plant	El Dorado, AR	621,000	2,200	0	0	623,200	0	3,080
Gerber Products Company	Fort Smith, AR	20,000	No Data	No Data	0	20,000	0	0
Ethyl Corporation	Magnolia, AR	83,000	0	0	0	83,000	0	0
	Hollister, CA	250	0	0	0	250	0	0
Asgrow Florida Company	Belle Glade, FL	1,850	0	0	0	1,850	0	0
Hms Chemicals Inc	Palmetto, FL	250	0	0	0	250	0	0
Florida Fertilizer Co. Inc.	Wauchula, FL	357	0	0	0	357	0	0
Hercules-Brunswick Plant	Brunswick, GA	72,900	0	0	0	72,900	0	0
Borden, Inc. Grocery & Specialty Products	Lowell, MA	18,200	No Data	0	0	18,200	No Data	No Data
Borden, Inc. Gorcery & Specialty Prds.	Warren, MI	14,200	No Data	0	0	14,200	No Data	0
Mobay Corporation - Agricultural Chemicals Div.	Kansas City, MO	11,500	0	0	0	11,500	0	0
Comet Delta, Inc.	Greenville, MS	21,300	0	0	0	21,300	0	0
Coastal Chemical Corporation	Greenville, NC	29	0	0	0	29	0	0
The Pillsbury Company	Buffalo, NY	12,000	No Data	0	0	12,000	0	No Data
Hershey Chocolate U.s.a. Hershey Plant	Hershey, PA	40,879	0	0	0	40,879	0	No Data
Consolidated Cigar Corp.	Cayey, PR	7,140	0	0	0	7,140	0	0
Amoco Chemical Company Cooper River	Wando, SC	220,000	0	0	0	220,000	0	0
Cargill Flour Milling	Saginaw, TX	30,000	0	0	0	30,000	0	0
Totals		1,305,855	2,200	0	0	1,308,055	0	3,080

[a]Derived from TRI 1989.
[b]POTW -- publicly-owned treatment works.

The ocean is another important source of bromomethane release to air. It seems likely that this is the result of bromomethane production by marine organisms, but the exact source is not known (IARC 1986). Singh et al. (1983b) calculated that the amount released from the ocean was large enough to account for most bromomethane in the atmosphere, but Penkett et al. (1985) showed that anthropogenic releases were the most important source. This is supported by the observation that atmospheric levels are about 40% higher in the northern hemisphere (0.015-0.026 ppb) than the southern hemisphere (0.011-0.019 ppb) (Penkett et al. 1985; Singh et al. 1983b).

Use of bromine-containing additives (ethylene dibromide) in leaded gasoline results in the release of bromomethane in exhaust fumes (about 70-220 $\mu g/m^3$ of exhaust) (Harsch and Rasmussen 1977), and this may have been a significant source of bromomethane release in the past. Combustion of unleaded gasoline releases much less bromomethane (about 4-5 $\mu g/m^3$), so current emissions from this source are presumably much lower than previously, and are likely to decrease further as leaded gasoline continues to be phased out.

5.2.2 Water

Because of its volatility, very little bromomethane is released to water. As shown in Table 5-1, no surface water releases were reported in the United States from industrial producers or processors of bromomethane (TRI 1989), although in one case about 2,000 pounds of bromomethane was released to groundwater through underground injection. Some bromomethane may leach from fumigated soil into surface water (EPA 1986b; IARC 1986). Most of this would be expected to quickly volatilize into air (see Section 5.3.1.), although some could migrate downward into groundwater where evaporation is not significant.

Bromomethane has not been detected in surface waters near any of 405 waste sites (including 99 NPL sites) where it was investigated, but it was detected in six groundwater samples from two locations (both NPL sites) (CLPSD 1989). The geometric mean of six samples from these two sites was 17 $\mu g/L$.

5.2.3 Soil

Soil fumigation is the primary use of bromomethane in the United States, accounting for approximately 65% of total consumption (EPA 1989c; IARC 1986). Based on reported production for 1984 (43 million pounds), this would be about 28 million pounds/year. However, as discussed in Section 5.3.1, most bromomethane will tend to evaporate from the soil within l-2 days, so soil contamination is normally not persistent. No industrial releases of bromomethane to soil were reported for 1987 (TRI 1989; see Table 5-l), and bromomethane has not been detected in soils or sediments at 455 hazardous waste sites, including 99 NPL sites (CLPSD 1989).

5.3 ENVIRONMENTAL FATE

5.3.1 Transport and Partitioning

Bromomethane is a readily volatile compound, with a boiling point of 3.6°C (Windholz 1983) and a vapor pressure at 20°C of 1,420 mmHg (Mabey et al. 1982). Consequently, bromomethane has a strong tendency to volatilize into air from other media (soil, water).

Because bromomethane is quite soluble in water (approximately 13-18 g/L) (EPA 1986b), some bromomethane in air may partition into clouds, rain, or surface waters. This tendency is described by the Henry's law constant (H), which for bromomethane has a value of 0.2 $atm \cdot m^3$/mole (Mabey et al. 1982). This value is sufficiently large to indicate that partitioning of bromomethane from air into water will be quite small. Conversely, the rate of bromomethane volatilization from water into air will be quite high, depending on mixing, temperature, and depth. The measured rate constant for volatilization is 22.5 cm/hr, which corresponds to a volatilization half-life of 3.1 hours for water 1 meter deep (Lyman et al. 1982). Half-lives of volatilization for lakes and deeper rivers range from 1 to 5 days (EPA 1986b). Rapid volatilization into indoor air would also be expected if contaminated water were used for showering, bathing, or cooking, but this has not been studied.

Bromomethane, either as a gas or dissolved in water, has relatively low affinity for soils. This has been established by direct observation (Brown and Rolston 1980; Chisholm and Koblitsky 1943; Fuhr et al. 1948), and is also expected on the basis of the relatively small K_{oc} (measured values range from 1 to 10) for this chemical (EPA 1986b; Roy and Griffin 1985). Volatilization of bromomethane from soil is also relatively rapid, with half-lives ranging from 0.2 to 0.5 days, depending on depth (Jury et al. 1984). Bromomethane is not expected to bioconcentrate in aquatic organisms because of its low octanol/water partition coefficient (K_{ow}) (estimated to be about 13) (Callahan et al. 1979). The bioconcentration factor (BCF) for bromomethane has not been measured experimentally. However, based on an empirical relation between the BCF and the K_{ow} (Neely et al. 1974), the estimated BCF for bromomethane is about 3. This low estimated BCF indicates that bromomethane should not significantly bioconcentrate (EPA 1986b).

5.3.2 Transformation and Degradation

5.3.2.1 Air

The main degradation pathway for bromomethane in air is reaction with photochemically-generated hydroxyl radicals. The rate constant for this reaction has been measured to be 4.14×10^{-14} $cm^3 \cdot molecule^{-1} \cdot sec^{-1}$ at 25°C, and 2.7×10^{-14} $cm^3 \cdot molecule^{-1} \cdot sec^{-1}$ at -8°C (the average temperature of the troposphere) (Davis et al. 1976). Assuming a concentration of atmospheric hydroxyl radicals of 9×10^5 molecules/cm^3, this corresponds to a tropospheric half-life of about 11 months. Thus, breakdown is relatively slow, and

bromomethane will tend to become widely dispersed in the atmosphere. Molecules that diffuse upward and reach the stratosphere may undergo direct photolytic degradation by ultraviolet radiation (Robbins 1976), but this degradation pathway accounts for only a small fraction (about 3%) of atmospheric bromomethane degradation (EPA 1986b).

5.3.2.2 Water

Bromomethane tends to undergo slow hydrolysis in water, yielding methanol, bromide ion, and hydrogen ion. The rate constant of this reaction has been measured to be about $3x10^{-7}$/second at 25°C (Castro and Belser 1981), and hydrolytic half-lives may range between 20 and 38 days, depending on temperature and pH (Castro and Belser 1981; Ehrenberg et al. 1974; Mabey and Mill 1978). It should be noted that these hydrolysis half-lives are considerably longer than typical volatilization half-lives (see Section 5.3.1). Thus, most bromomethane will volatilize from water before extensive hydrolysis occurs.

5.3.2.3 Soil

The principal fate of bromomethane in soil is volatilization, but some may react with organic soil constituents to yield nonvolatile end products, including bromide ion (Brown and Rolston 1980; Goring et al. 1975; Shiroishi et al. 1964). There is little evidence that bromomethane in soil is degraded by microorganisms (EPA 1986b).

5.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

5.4.1 Air

As shown in Table 5-2, bromomethane has been detected in air samples from regions all around the globe. Concentrations over the oceans and in rural areas are typically less than 0.025 ppb (0.1 $\mu g/m^3$), while concentrations in suburban and urban areas may range up to 1.2 ppb (5 $\mu g/m^3$). These values are all much lower than may be encountered near places where bromomethane is being used for fumigation (25 ppm or 100,000 $\mu g/m^3$) (Bond and Durnas 1987).

5.4.2 Water

Bromomethane occurs in ocean waters at a concentration of about l-2 ng/L (Lovelock 1975; Singh et al. 1983b), but is not a common contaminant in fresh waters in the United States. It was not detected in storm water runoff from 15 U.S. cities (Cole et al. 1984) or in influents to sewage treatment plants in four cities (Levins et al. 1979), and was detected in only 1.4% of over 900 surface water samples recorded in the STORET database (Staples et al. 1985). The median concentration in these positive samples was less than 10 $\mu g/L$.

TABLE 5-2. Summary of Bromomethane Levels in Air

Location	Concentration ($\mu g/m^3$)[a] Maximum	Mean	References
Northern Hemisphere	No data	0.02-0.08	Penkett et al. 1985; Singh et al. 1979b
Arctic	0.09	0.043	Berg et al. 1984
Oceanic	No data	0.09	Singh et al. 1983b
Rural/Suburban United States	No data	0.002-0.32[b]	Brodzinsky and Singh 1983; Harsch and Rasmussen 1977; Shah and Heyerdahl 1988
Urban United States	0.25-5.1	0.16-2.2	Brodzinsky and Singh 1983; Harsch and Rasmussen 1977; Shah and Heyerdahl 1988; Shikiya et al. 1984; Singh et al. 1981b, 1982
Source dominated[c]	1.1×10^5	No data	Bond and Dumas 1987
Hazardous waste sites	No data	-[d]	La Regina et al. 1986

[a]1 $\mu g/m^3$ = 0.25 ppb (0.00025 ppm).
[b]Median value.
[c]Data measured 25 m from a flour mill being fumigated with bromomethane.
[d]Detected, but not quantified; detection limit ≈0.4 $\mu g/m^3$.

5. POTENTIAL FOR HUMAN EXPOSURE

Bromomethane has been detected (but not quantified) in drinking water supplies of several U.S. cities (Coleman et al. 1976; EPA 1975; Kool et al. 1982; Kopfler et al. 1977; Shackelford and'Keith 1976). Bromomethane in drinking water is presumably generated as an inadvertent byproduct following chlorination.

Occurrence of bromomethane in groundwater is somewhat more likely than in surface water, since evaporation is restricted. Bromomethane has been detected in groundwater in New Jersey (Greenberg et al. 1982) but not in Wisconsin (Krill and Sonzogni 1986).

5.4.3 Soil

No data were found on bromomethane levels in soil. Bromomethane is not expected to be a stable constituent of soil, since it either .evaporates or reacts.with organic soil components (see Section 5.3.2.3). However, bromide ion may be retained in fumigated soil (IARC 1986). Bromomethane was not detected in 353 sediment samples from STORET stations in the United States (Staples et al. 1985).

5.4.4 Other Environmental Media

Although bromomethane is used extensively as a fumigant for grains and other food products, it is rarely detected unchanged as a residue in foods. Most of the fumigant is rapidly lost to the atmosphere, and the remaining portion reacts with the food components, producing residues of inorganic bromide (IARC 1986; NAS 1978). Daft (1987, 1988, 1989) and Cova et al. (1986) reported that bromomethane was not detected in hundreds of food products, and Duggan et al. (1983) found-bromomethane in only 3 of 5,631 samples of vegetables. The tolerances for residues on agricultural commodities and processed foods that have been set by EPA and FDA are for bromide ion, not bromomethane (21 CFR 193; 40 CFR 180).

5.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

Inhalation of bromomethane in ambient air is the predominant exposure route for most people in the United States. Singh et al. (1981b) calculated that average daily doses of bromomethane from air in 3 U.S. cities ranged from 4.5 to 24.5 μg/person, based on total air intake of 23 m^3/day by an adult. These estimates were based on 1979 monitoring data in urban areas. It is likely that urban bromomethane levels are currently lower than in the past, due to decreased emissions from automobiles using leaded gasoline (see Section 5.2.1). Based on the very low levels of bromomethane in water and the negligible levels in food, it appears that exposure of the general population to bromomethane from sources other than air is likely to be insignificant under normal circumstances.

Exposure of workers to bromomethane is highly variable, depending on conditions. Exposure levels inside factories are regulated by OSHA, and the 8-hour average concentration is not permitted to exceed 5 ppm (OSHA 1989).

Highest exposures are most likely to occur during fumigation activities, especially when bromomethane is first released to the environment after fumigation ends. Exposure levels under these conditions could reach from 25 to 2,500 ppm (IARC 1986; NIOSH 1984a; Van Den Oever et al. 1982), which would correspond to a dose of 100-10,000 mg/hour for an exposed worker. NIOSH estimated that about 105,000 workers in the United States were potentially exposed to bromomethane in the workplace in 1980 (NIOSH 1984a).

5.6 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Members of the general population are not likely to be exposed to high levels of bromomethane except in the immediate vicinity of industrial facilities that release the gas into air, or near locations where bromomethane is being used as a soil or a space fumigant. This includes individuals returning to work or live in locations that have recently been fumigated, especially if insufficient time has been allowed for the chemical to disperse. Individuals living near waste sites that contain bromomethane might also be exposed, although the level of exposure is not known. Individuals involved in the production of bromomethane and those licensed to use it as a fumigant may be exposed to high levels if proper safety precautions are not followed.

5.7 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of bromomethane is available. Where adequate information is not available, ATSDR, in conjunction with the NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of bromomethane.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that, if met, would reduce or eliminate the uncertainties of human health assessment. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

5.7.1 Data Needs

Physical and Chemical Properties. The physical and chemical properties of bromomethane are sufficiently well known to allow estimation of environmental fate. Although there is some disparity in reported values for the solubility in water and Henry's law constant for bromomethane (see Table 3-l), further studies to define these parameters more precisely do not appear essential, since volatilization from water is so rapid.

5. POTENTIAL FOR HUMAN EXPOSURE

Production, Import/Export, Use, and Disposal. Large quantities of bromomethane are produced and used in this country (TRI 1989), and there is significant opportunity for humans to be exposed to both during production and use (IARC 1986). Information is available on current volumes, and available data suggest production is increasing (IARC 1986). The main use of bromomethane is as a fumigant for soil, agricultural produce, and structures (IARC 1986), but data on the amount of bromomethane used for each type of fumigation were not located. Due to its volatility, nearly all releases from fumigation are to air (TRI 1989), and this is the medium most likely to be contaminated. Currently there are no regulations which restrict this release.

According to the Emergency Planning and Community Nght-to-Know Act of 1986, 42 U.S.C. Section 11023, industries are required to submit chemical release and off-site transfer information to the EPA. The Toxics Release Inventory (TRI), which contains this information for 1987, became available in May of 1989. This database will be updated yearly and should provide a list of industrial production facilities and emissions.

Environmental Fate. The fate of bromomethane in the environment is dominated by rapid evaporation into air, where it is quite stable (EPA 1986b). The rates of volatilization from soil and water have been studied and are known with reasonable precision (although such rates are typically sitespecific) (Jury et al. 1984; Lyman et al. 1982). The rates of breakdown by hydrolysis, reaction with hydroxyl radical, and direct photolysis in the stratosphere have also been estimated (Castro and Belser 1981; Davis et al. 1976; Robbins 1976). Further studies to improve the accuracy of available rate constants for these processes would be helpful, but do not appear to be essential in understanding the basic behavior of bromomethane in the environment.

Bioavailability from Environmental Media. Bromomethane is known to be well absorbed following inhalation and oral contact (Gargas and Andersen 1982; Medinsky et al. 1984). Small amounts may also be absorbed across the skin, but this has not been quantified. No information was located regarding the relative bioavailability of bromomethane from media such as food or soil. However, since bromomethane has a low K_{oc} value (Mabey et al. 1982), it is not likely that bioavailability would be much reduced by these matrices. Moreover, since bromomethane is rarely found in these media, research on this subject does not appear essential.

Food Chain Bioaccumulation. Although the bioconcentration, bioaccumulation, and biomagnification of bromomethane have not been formally investigated, it seems clear that these are not of significant concern. This is the result of several factors, including the high volatility and high water solubility of the compound, its low K_{ow}, and its relatively rapid metabolism by reaction with organic materials (Mabey et al. 1982; Medinsky et al. 1985). On this basis, it does not appear that research in this area is essential.

Exposure Levels in Environmental Media. Several studies are available documenting bromomethane concentrations in ambient air (Brodzinsky and Singh 1983; Harsch and Rasmussen 1977), but data for bromomethane in water are rare. Bromomethane has been analyzed for, but rarely detected, in foods (Daft 1987, 1988, 1989). Human exposure levels of bromomethane by inhalation of urban air have been calculated (Singh et al. 1981b). However, these levels are based on monitoring data more than 10 years old. Since urban air concentrations of bromomethane may have decreased due to reduced emissions from automobiles, exposure levels calculated from past data should be taken as an upper limit, and new levels calculated from current monitoring data would be useful. Additional monitoring data on levels in air near sites where bromomethane is being made or used would also be valuable in defining environmental levels.

Exposure Levels in Humans. Bromomethane is not normally measured in human tissues such as blood or urine, even in people exposed to high levels. This is because bromomethane is removed from the body very quickly after exposure ceases. Consequently, this is not likely to be a useful means of monitoring exposure of humans to low levels of bromomethane. Increased levels of bromide have been detected in blood of persons exposed to bromomethane in accidents or in the workplace, but no studies were located regarding bromide levels in persons potentially exposed to bromomethane near waste sites. Since bromide is a normal component of serum, and since the serum bromide level is quite variable, it does not seem that broad surveys of blood bromide levels in persons living near waste sites would be useful. However, site-specific studies at locations where bromomethane exposure is likely might prove helpful.

Exposure Registries. No exposure registries for bromomethane were located. This compound is not currently one of the compounds for which a subregistry has been established in the National Exposure Registry. The compound will be considered in the future when chemical selection is made for subregistries to be established. The information that is amassed in the National Exposure Registry facilitates the epidemiological research needed to assess adverse health outcomes that may be related to the exposure to this compound.

5.7.2 On-going Studies

No information was located on any on-going studies on the fate and transport of bromomethane. However, two studies related to human exposure to bromomethane are being supported by the U.S. Department of Agriculture and conducted at the University of California, Davis. One project will analyze bromomethane residues on foods, and the second will quantitate exposure levels of field workers to bromomethane and develop appropriate procedures to minimize exposure from this source. Remedial investigations and feasibility studies at NPL sites that contain bromomethane will provide further information on environmental concentrations and human exposure levels near waste sites.

6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting and/or measuring and monitoring bromomethane in environmental media and in biological samples. The intent is not to provide an exhaustive list of analytical methods that could be used to detect and quantify bromomethane. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used to detect bromomethane in environmental samples are the methods approved by federal agencies such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that refine previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

As a volatile material, bromomethane is readily determined by gas chromatographic analysis. The selectivity and sensitivity of detection are increased by the use of an electron capture detector or a halide-specific detector, both of which are very sensitive for organohalides such as bromomethane. Specificity in detection is achieved with mass spectrometric detectors.

6.1 BIOLOGICAL MATERIALS

Bromomethane may be isolated from biological materials either by extraction into an organic solvent, or simply by collecting headspace vapors. Table 6-l summarizes several methods used by researchers for measuring parent bromomethane in blood or tissues. Detection limits are sufficiently low that levels in blood or tissue associated with health effects can easily be measured. However, as discussed in Section 2.3.4, parent bromomethane is cleared from blood and tissues quite rapidly, so detection of bromomethane exposure in humans is typically performed by measuring serum bromide levels instead. Several methods for measuring bromide ion in serum are also presented in Table 6-l. These methods are also sufficiently sensitive that detection limits (0.5-2.5 ppm) are lower than typical levels of bromide in serum of unexposed people (5-15 ppm), and increases due to bromomethane exposure can easily be measured (Alexeeff and Kilgore 1983).

6.2 ENVIRONMENTAL SAMPLES

Collection of bromomethane from environmental samples is nearly always achieved by trapping on a solid sorbent such as activated charcoal. For air samples, this is done simply by drawing the air through the sorbent. For water, soil, or solid wastes, bromomethane is purged from the sample by flushing with an inert gas, and this is then passed through the sorbent. Desorption may be achieved by extraction in a convenient solvent, or by heating. Table 6-2 summarizes a number of methods that have been developed for measuring bromomethane in various types of environmental media. In all cases, detection limits are much lower than levels of health concern.

TABLE 6-1. Analytical Methods for Determining Bromomethane in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Bromomethane					
Blood, tissue	Purge with inert gas, trap on Tenax® GC, desorb thermally[a]	GC/MS	3 ng/mL blood 6 ng/mL tissue	No data	Pellizzari et al. 1985
Blood, tissue, adipose	Homogenize in toluene, centrifuge, inject supernatant fluid	GC/ECD	1 ng/g	100	Honma et al. 1985
Food	Collect headspace vapor	HRGC	0.4 ppb	No data	DeVries et al. 1985
Grain	Extract with acetone, collect headspace vapor from acetone extract	GC	<1 mg/kg	91.3±3	Scudamore 1985
Bromide					
Blood plasma	Collect headspace vapor of HBr from plasma treated with dimethyl sulfate at 85°C	HRGC	<0.5 μg/mL bromide	97.3±6.3 at 5 μg/mL	Yamano et al. 1987
Tissues	Extract in 18% trichloroacetic acid; derivatize to 1,2-dibromo-cylcohexanone	GC/ECD	2.5 μg/g	17	Honma et al. 1985
Serum, urine	Digest in KOH. Convert to bromate, then to tetrabromorosaniline	Colorimetric (570 nm)	1 μg/mL	100±1	Hunter 1955

[a]Method for the determination of volatile halocarbons in blood and tissue adaptable to bromomethane determination.

ECD = electron capture detector; GC = gas chromatography; HRGC = high-resolution gas chromatography; MS = mass spectrometry

TABLE 6-2. Analytical Methods for Determining Bromomethane in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Sorption by activated carbon, desorption by carbon disulfide	GC/FID	<20 mg/m³	No data	Mackenzie Peers 1985
Air	Sorption by HBr-treated activated carbon, desorption with carbon disulfide	GC/ECD	0.2 ppm	No data	LeFevre et al. 1989
Air	Retention by activated carbon, removal as headspace gas	GC/ECD	2 μg/m³	37-91	Woodrow et al. 1988
Exhaust gas	Collect on Tenax® GC, desorb	GC/MWP	<90 μg/m³	No data	Baumann and Heumann 1987
Water	Purge with inert gas, trap on sorbent trap, desorb thermally	GC/HSD	Approx. 0.5 μg/L	No data	APHA 1985a
Water	Purge with inert gas, trap on sorbent trap, desorb thermally	GC/MS	<1 μg/L	96±11	APHA 1985b
Water	Purge with inert gas, trap on sorbent trap, desorb thermally	GC/HSD	0.01 μg/L	90-110±10[a]	EPA 1988d
Water	Purge with inert gas, trap on sorbent trap, desorb thermally	HRGC/HSD	0.01-0.05 μg/L	97±4	EPA 1988e
Water	Purge with inert gas, trap on sorbent trap, desorb thermally	HRGC/MS	0.11 μg/L	95±8	EPA 1988g
Wastewater	Purge with inert gas, trap on sorbent trap, desorb thermally	GC/MS	No data	88±23	EPA 1982b
Solid waste	Purge by helium, collect on solid, thermally desorb	GC/MS	10 μg/kg	111±48 at 37.2 μg/L	EPA 1986c

[a]Value for similar volatile organohalide compounds in air, not determined directly for bromomethane.

ECD = electron capture detector; FID = flame ionization detector; GC = gas chromatography; HRGC = high-resolution gas chromatography; HSD = haldie specific detector; MS = mass spectrometry; MWP = microwave plasma

6.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of bromomethane is available. Where adequate information is not available, ATSDR, in conjunction with the NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of bromomethane.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that, if met, would reduce or eliminate the uncertainties of human health assessment. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.3.1 Data Needs

Methods for Determining Biomarkers of Exposure and Effect. Exposure to bromomethane may be evaluated by measuring parent bromomethane, serum bromide, or methylated adducts. Existing methods can measure parent bromomethane in blood or expired air with excellent sensitivity (Honma et al. 1985; Pellizzari et al. 1985; Woodrow et al. 1988), but this is rarely done because bromomethane is cleared so quickly. Several sensitive methods exist for measuring serum bromide (Honma et al. 1985; Hunter 1955; Yamano et al. 1987), and this is the most common means for evaluating exposure. However, increased bromide is not specific for bromomethane exposure, and levels may vary widely between individuals. No routine methods have been established for measuring methyl adducts in DNA or protein (except those involving ^{14}C-labeled bromomethane). Efforts to develop sensitive and specific immunoassays for these adducts would be valuable, since levels of these adducts may be directly proportional to tissue damage. In addition, combination of a method for detecting methyl adducts with a test for increased serum bromide would increase specificity in bromomethane exposure estimates.

The characteristic markers of effect in people exposed to high levels of bromomethane are lung irritation, renal shut-down, and central nervous system injury (Clarke et al. 1945; O'Neal 1987; Prain and Smith 1952). In people exposed to low levels, only the neurological effects can be detected (Anger et al. 1986; Kishi et al. 1988; Verberk et al. 1979). Other than standard clinical neurological or neurobehavior tests, no specific biomarkers of bromomethane effects are known. Since parameters measured in these tests are highly variable among individuals, these tests are neither specific nor particularly sensitive. Efforts to identify and develop a more specific and objective biomarker of exposure would be valuable in evaluating the health significance of exposures that might occur in the environment or near waste sites.

6. ANALYTICAL METHODS

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. The medium of main concern for human exposure to bromomethane is air. Trace levels may occur in water or soil, but human exposures from these sources are not expected to be large enough to be of concern except in rare situations. Existing analytical methods can measure bromomethane in air (LeFevre et al. 1989; Mackenzie Peers 1985; Woodrow et al. 1988) and other environmental media (APHA 1985a, 1985b; EPA 1982b, 1986c, 1988d, 1988e, 19883) at levels considerably below those of health concern. The accuracy and precision of the methods are established, and adequate specificity may be achieved by use of mass spectrophotometric detectors. Nevertheless, further efforts to improve accuracy and ease of sample isolation and transfer to the analytical instrument would be helpful.

6.3.2 On-going Studies

The Environmental Health Laboratory Sciences Division of the Center for Environmental Health and Injury Control, Centers for Disease Control, is developing methods for the analysis of bromomethane and other volatile organic compounds in blood. These methods use purge and trap methodology and magnetic mass spectrometry which gives detection limits in the low parts per trillion range.

Research is underway at the Cooperative Institute for Research in Environmental Sciences (CIRES) at the University of Colorado, Boulder, to improve methods of analysis for bromomethane and related compounds in environmental samples.

Examination of the literature suggests that studies are in progress to improve means for determining bromomethane, its metabolites, and related compounds in biological samples and environmental media. For example, a "Master Analytical Scheme" is being developed for organic compounds in water (Michael et al. 1988), which includes bromomethane as an analyte. The overall goal is to detect and quantitatively measure organic compounds at 0.1 μg/L in drinking water, 1 μg/L in surface waters, and 10 μg/L in effluent waters. Improvements continue to be made in chromatographic separation and detection. Problems associated with the collection of bromomethane on a sorbent trap, followed by thermal desorption may be overcome with direct purging to a capillary column with whole column cryotrapping (Pankow and Rosen 1988). Current research activities in supercritical fluid extraction (King 1989) and supercritical fluid chromatography (Smith 1988) include organohalide analytes such as bromomethane in biological samples and environmental media.

7. REGULATIONS AND ADVISORIES

Because of its potential to cause adverse health effects in exposed people, a number of regulations and guidelines have been established for bromomethane by various national and state agencies. These values are summarized in Table 7-l.

7. REGULATIONS AND ADVISORIES

TABLE 7-1. Regulations and Guidelines Applicable to Bromomethane

Agency	Description	Information	References
INTERNATIONAL			
IARC	Carcinogenic classification	Group 3[a]	IARC 1987
NATIONAL			
Regulations:			
a. Air:			
OSHA	PEL TWA	5 ppm (20 mg/m^3), skin	OSHA 1989 (29 CFR 1910.1000) Table Z-1-A
b. Water:			
EPA ODW	Monitoring requirements for unregulated contaminants	Yes	EPA 1987b (40 CFR 142)
EPA OWRS	General permits under NPDES	Yes	40 CFR 122, Appendix D, Table II
	General Pretreatment Regulations for Existing and New Sources of Pollution	Yes	40 CFR 403
	Hazardous substance	Yes	40 CFR 116
	Reportable quantity	1,000 pounds	40 CFR 117.3
c. Food:			
EPA OPP	Tolerances for residues of inorganic bromides resulting from fumigation with methyl bromide in or on raw agricultural commodities	5-240 ppm	40 CFR 180.123
	Tolerances for residues of inorganic bromide resulting from soil treatment with combinations of chloropicrin, methyl bromide and propargyl bromide	25-300 ppm	40 CFR 180.199
FDA	Tolerances of inorganic bromide in processed food as a result of fumigation with methyl bromide	125-400 ppm	21 CFR 193.250
	Tolerances for residues of inorganic bromide from fumigation with methyl bromide on cereal grains and processed grains used in production of fermented malt beverages	125 ppm	21 CFR 193.225, 193.230
d. Other:			
EPA OERR	Reportable quantity	1,000 pounds	EPA 1989a,b 40 CFR 302.4)
	Extremely Hazardous Substance Threshold Planning Quantity	1,000 pounds	EPA 1987a (40 CFR 355)
EPA OPP	Restricted use pesticide	Yes	40 CFR 162.31

7. REGULATIONS AND ADVISORIES

TABLE 7-1 (Continued)

Agency	Description	Information	References
NATIONAL (Cont.)			
EPA OSW	Hazardous Waste Constituent (Appendix VIII)	Yes	EPA 1980b (40 CFR 261)
	Groundwater monitoring list (Appendix IX)	Yes	EPA 1987c (40 CFR 264)
	Land disposal restrictions	Yes	EPA 1987d, 1988b (40 CFR 268)
EPA OTS	Toxic chemical release reporting rule	Yes	EPA 1988a (40 CFR 372)
	Health and safety data reporting rule	Yes	EPA 1988c (40 CFR 716.120)
Guidelines:			
a. Air:			
ACGIH	TLV TWA	5 ppm (19 mg/m^3)	ACGIH 1991
NIOSH	IDLH	2,000 ppm	NIOSH 1990
	REL	carcinogen; lowest feasible concentration	
b. Water:			
EPA OWRS	Ambient Water Quality Criteria		EPA 1980a
	Ingesting water and organisms:	1.9×10^{-4} mg/L[b]	
	Ingesting organisms only:	1.57×10^{-2} mg/L[b]	
	For noncarcinogenic effects	1.4 mg/L	
c. Other:			
EPA	Carcinogenic Classification	Group D[c]	EPA 1989c,
	Oral RfD	1.4×10^{-3} mg/kg/day	IRIS 1989
STATE			
Regulations and Guidelines:			
a. Air:	Acceptable ambient air concentrations		NATICH 1989
Connecticut		400 μg/m^3 (8 hr)	
Kansas		47.6 μg/m^3 (annual)	
Massachusetts		2.6 μg/m^3 (24 hr)	
Nevada		0.476 mg/m^3 (8 hr)	
North Dakota		0.20 mg/m^3 (8 hr)	
Pennsylvania (Philadelphia)		480 μg/m^3 (1 yr)	
South Carolina		100 μg/m^3 (24 hr)	
Vermont		0.01 μg/m^3 (annual)	
Virginia		350 μg/m^3 (24 hr)	
b. Water:	Drinking water quality standards		FSTRAC 1988
Arizona		2.5 μg/L	
Kansas		0.19 μg/L	
Massachusetts		0.01 mg/L	ORS 1989

[a]Group 3: Not classifiable as to carcinogenic potential.
[b]Values for incremental lifetime cancer risk of 10^{-6} for halomethanes as a class based on carcinogenicity of chloroform.
[c]Group D: Not classifiable as to human carcinogenicity.

ACGIH = American Conference of Governmental Industrial Hygienists; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; IARC = International Agency for Research on Cancer; IDLH = Immediately Dangerous to Life or Health Level; NIOSH = National Institute for Occupational Safety and Health; NPDES = National Pollutant Discharge Elimination System; ODW = Office of Drinking Water; OERR = Office of Emergency and Remedial Response; OPP = Office of Pesticide Products; OSHA = Occupational Safety and Health Administration; OSW = Office of Solid Wastes; OTS = Office of Toxic Substances; OWRS = Office of Water Regulations and Standards; PEL = Permissible Exposure Limit; REL = recommended exposure limit; RfD = reference dose; TLV = Threshold Limit Value; TWA = Time-Weighted Average

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API. 1989c. American Petroleum Institute Statistics Department. Latest API statistics: Week ending October 6, 1989. The Oil Daily, October 13, 1989.

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9. GLOSSARY

Acute Exposure -- Exposure to a chemical for a duration of 14 days or less, as specified in the Toxicological Profiles.

Adsorption Coefficient (K_{oc}) -- The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

Adsorption Ratio (K_d) -- The amount of a chemical adsorbed by a sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

Bioconcentration Factor (BCF) -- The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

Cancer Effect Level (CEL) -- The lowest dose of chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or tumors) between the exposed population and its appropriate control.

Carcinogen -- A chemical capable of inducing cancer.

Ceiling Value -- A concentration of a substance that should not be exceeded, even instantaneously.

Chronic Exposure -- Exposure to a chemical for 365 days or more, as specified in the Toxicological Profiles.

Developmental Toxicity -- The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to'the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Embryotoxicity and Fetotoxicity -- Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the insult occurred. The terms, as used here, include malformations and variations, altered growth, and in utero death.

EPA Health Advisory -- An estimate of acceptable drinking water levels for a chemical substance based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

Immediately Dangerous to Life or Health (IDLH) -- The maximum environmental concentration of a contaminant from which one could escape within 30 min without any escape-impairing symptoms or irreversible health effects.

Intermediate Exposure -- Exposure to a chemical for a duration of 15-364 days as specified in the Toxicological Profiles.

Immunologic Toxicity -- The occurrence of adverse effects on the immune system that may result from exposure to environmental agents such as chemicals.

In Vitro -- Isolated from the living organism and artificially maintained, as in a test tube.

In Vivo -- Occurring within the living organism.

Lethal Concentration($_{Lo}$) (LC_{Lo}) -- The lowest concentration of a chemical in air which has been reported to have caused death in humans or animals.

Lethal Concentration($_{50}$) (LC_{50}) -- A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose($_{Lo}$) (LD_{Lo}) -- The lowest dose of a chemical introduced by a route other than inhalation that is expected to have caused death in humans or animals.

Lethal Dose($_{50}$) (LD_{50}) -- The dose of a chemical which has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time($_{50}$) (LT_{50}) -- A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL) -- The lowest dose of chemical in a study or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Malformations -- Permanent structural changes that may adversely affect survival, development, or function.

Minimal Risk Level -- An estimate of daily human exposure to a chemical that is likely to be without an appreciable risk of deleterious effects (noncancerous) over a specified duration of exposure.

Mutagen -- A substance that causes mutations. A mutation is a change in the genetic material in a body cell. Mutations can lead to birth defects, miscarriages, or cancer.

Neurotoxicity -- The occurrence of adverse effects on the nervous system following exposure to chemical.

9. GLOSSARY

No-Observed-Adverse-Effect Level (NOAEL) -- The dose of chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Effects may be produced at this dose, but they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{ow}) -- The equilibrium ratio of the concentrations of a chemical in n-octanol and water, in dilute solution. Permissible Exposure Limit (PEL) -- An allowable exposure level in workplace air averaged over an g-hour shift.

q_1* -- The upper-bound estimate of the low-dose slope of the dose-response curve as determined by the multistage procedure. The q_1* can be used to calculate an estimate of carcinogenic potency, the incremental excess cancer risk per unit of exposure (usually μg/L for water, mg/kg/day for food, and μg/m^3 for air).

Reference Dose (RfD) -- An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure of the human population to a potential hazard that is likely to be without risk of deleterious effects during a lifetime. The RfD is operationally derived from the NOAEL (from animal and human studies) by a consistent application of uncertainty factors that reflect various types of data used to estimate RfDs and an additional modifying factor, which is based on a professional judgment of the entire database on the chemical. The RfDs are not applicable to nonthreshold effects such as cancer.

Reportable Quantity (RQ) -- The quantity of a hazardous substance that is considered reportable under CERCLA. Reportable quantities are: (1) 1 lb or greater or (2) for selected substances, an amount established by regulation either under CERCLA or under Sect. 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

Reproductive Toxicity -- The occurrence of adverse effects on the reproductive system that may result from exposure to a chemical. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

Short-Term Exposure Limit (STEL) -- The maximum concentration to which workers can be exposed for up to 15 min continually. No more than four excursions are allowed per day, and there must be at least 60 min between exposure periods. The daily TLV-TWA may not be exceeded.

Target Organ Toxicity -- This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

9. GLOSSARY

Teratogen -- A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV) -- A concentration of a substance to which most workers can be exposed without adverse effect. The TLV may be expressed as a TWA, as a STEL, or as a CL.

Time-weighted Average (TWA) -- An allowable exposure concentration averaged over a normal 8-hour workday or 40-hour workweek.

Toxic Dose (TD_{50}) -- A calculated dose of a chemical, introduced by a route other than inhalation, which is expected to cause a specific toxic effect in 50% of a defined experimental animal population.

Uncertainty Factor (UF) -- A factor used in operationally deriving the RfD from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using LOAEL data rather than NOAEL data. Usually each of these factors is set equal to 10.

APPENDIX A

USER'S GUIDE

Chapter 1

Public Health Statement

This chapter of the profile is a health effects summary written in nontechnical language. Its intended audience is the general public especially people living in the vicinity of a hazardous waste site or substance release. If the Public Health Statement were removed from the rest of the document, it would still communicate to the lay public essential information about the substance.

The major headings in the Public Health Statement are useful to find specific topics of concern. The topics are written in a question and answer format. The answer to each question includes a sentence that will direct the reader to chapters in the profile that will provide more information on the given topic.

Chapter 2

Tables and Figures for Levels of Significant Exposure (LSE)

Tables (2-1, 2-2, and 2-3) and figures (2-l and 2-2) are used to summarize health effects by duration of exposure and endpoint and to illustrate graphically levels of exposure associated with those effects. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of No-Observed-Adverse-Effect Levels (NOAELS), Lowest-Observed- Adverse-Effect Levels (LOAELs) for Less Serious and Serious health effects, or Cancer Effect Levels (CELs). In addition, these tables and figures illustrate differences in response by species, Minimal Risk Levels (MRLs) to humans for noncancer end points, and EPA's estimated range associated with an upper-bound individual lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. The LSE tables and figures can be used for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text.

The legends presented below demonstrate the application of these tables and figures. A representative example of LSE Table 2-l and Figure 2-l are shown. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

LEGEND

See LSE Table 2-l

(1) Route of Exposure One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. When sufficient data exist,

three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure, i.e., inhalation, oral, and dermal (LSE Table 2-1, 2-2, and 2-3, respectively). LSE figures are limited to the inhalation (LSE Figure 2-1) and oral (LSE Figure 2-2) routes.

(2) Exposure Duration Three exposure periods: acute (14 days or less); intermediate (15 to 364 days); and chronic (365 days or more) are presented within each route of exposure. In this example, an inhalation study of intermediate duration exposure is reported.

(3) Health Effect The major categories of health effects included in LSE tables and figures are death, systemic, immunological, neurological, developmental, reproductive, and cancer. NOAELs and LOAELs can be reported in the tables and figures for all effects but cancer. Systemic effects are further defined in the "System" column of the LSE table.

(4) Key to Figure Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 18 has been used to define a NOAEL and a Less Serious LOAEL (also see the two "18r" data points in Figure 2-l).

(5) Species The test species, whether animal or human, are identified in this column.

(6) Exposure Freauencv/Duration The duration of the study and the weekly and daily exposure regimen are provided in this column. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 18), rats were exposed to [substance x] via inhalation for 13 weeks, 5 days per week, for 6 hours per day.

(7) System This column further defines the systemic effects. These systems include: respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, and dermal/ocular. "Other" refers to any systemic effect (e.g., a decrease in body weight) not covered in these systems. In the example of key number 18, one systemic effect (respiratory) was investigated in this study.

(8) NOAEL A No-Observed-Adverse-Effect Level (NOAEL) is the highest exposure level at which no harmful effects were seen in the organ system studied. Key number 18 reports a NOAEL of 3 ppm for the respiratory system which was used to derive an intermediate exposure, inhalation MRL of 0.005 ppm (see footnote "c").

(9) LOAEL A Lowest-Observed-Adverse-Effect Level (LOAEL) is the lowest exposure level used in the study that caused a harmful health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific end point used to

quantify the adverse effect accompani.es the MAEL. The "Less Serious" respiratory effect reported in key number 18 (hyperplasia) occurred at a LOAEL of 10 ppm.

(10). Reference The complete reference citation is given in Chapter 8 of the profile.

(11). CEL A Cancer Effect Level (CEL) is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiological studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses which did not cause a measurable increase in cancer.

(12). Footnotes Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. Footnote "c" indicates the NOAEL of 3 ppm in key number 18 was used to derive an MRL of 0.005 ppm.

LEGEND

See LSE Figure 2-l

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure levels for particular exposure duration.

(13). Exposure Duration The same exposure periods appear as in the LSE table. In this example, health effects observed within the intermediate and chronic exposure periods are illustrated.

(14). Health Effect These are the categories of health effects for which reliable quantitative data exist. The same health effects appear in the LSE table.

(15). Levels Of Exposure Exposure levels for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure levels are reported on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(16). NOAEL In this example, 18r NOAEL is the critical end point for which an intermediate inhalation exposure MRL is based. As you can see from the LSE figure key, the open-circle symbol indicates a NOAEL for the test species (rat). The key number 18 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 3 ppm (see entry 18 in the Table) to the MRL of 0.005 ppm (see footnote "b" in the LSE table).

(17). CEL Key number 38r is one of three studies for which Cancer Effect Levels (CELs) were derived. The diamond symbol refers to a CEL for the test species (rat). The number 38 corresponds to the entry in the LSE table.

(18). Estimated Upper-Bound Human Cancer Risk Levels This is the range associated with the upper-bound for lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. These risk levels are derived from EPA's Human Health Assessment Group's upper-bound estimates of the slope of the cancer dose response curve at low dose levels (q_1*).

(19). Key to LSE Figure The Key explains the abbreviations and symbols used in the figure.

SAMPLE

1 → **TABLE 2-1. Levels of Significant Exposure to [Chemical x] - Inhalation**

Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
2 → INTERMEDIATE EXPOSURE							
3 → Systemic	5	6	7	8	9		10
4 → 18	Rat	13 wk 5d/wk 6hr/d	Resp	3[b]	10 (hyperplasia)		Nitschke et al. 1981
CHRONIC EXPOSURE							
Cancer						11	
38	Rat	18 mo 5d/wk 7hr/d				20 (CEL, multiple organs)	Wong et al. 1982
39	Rat	89-104 wk 5d/wk 6hr/d				10 (CEL, lung tumors, nasal tumors)	NTP 1982
40	Mouse	79-103 wk 5d/wk 6hr/d				10 (CEL, lung tumors, hemangiosarcomas)	NTP 1982

[a] The number corresponds to entries in Figure 2-1.

12 → [b] Used to derive an intermediate inhalation Minimal Risk Level (MRL) of 5×10^{-3} ppm; dose adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animal to humans, 10 for human variability).

CEL = cancer effect level; d = day(s); hr = hour(s); LOAEL = lowest-observed-adverse-effect level; mo = month(s); NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s)

SAMPLE

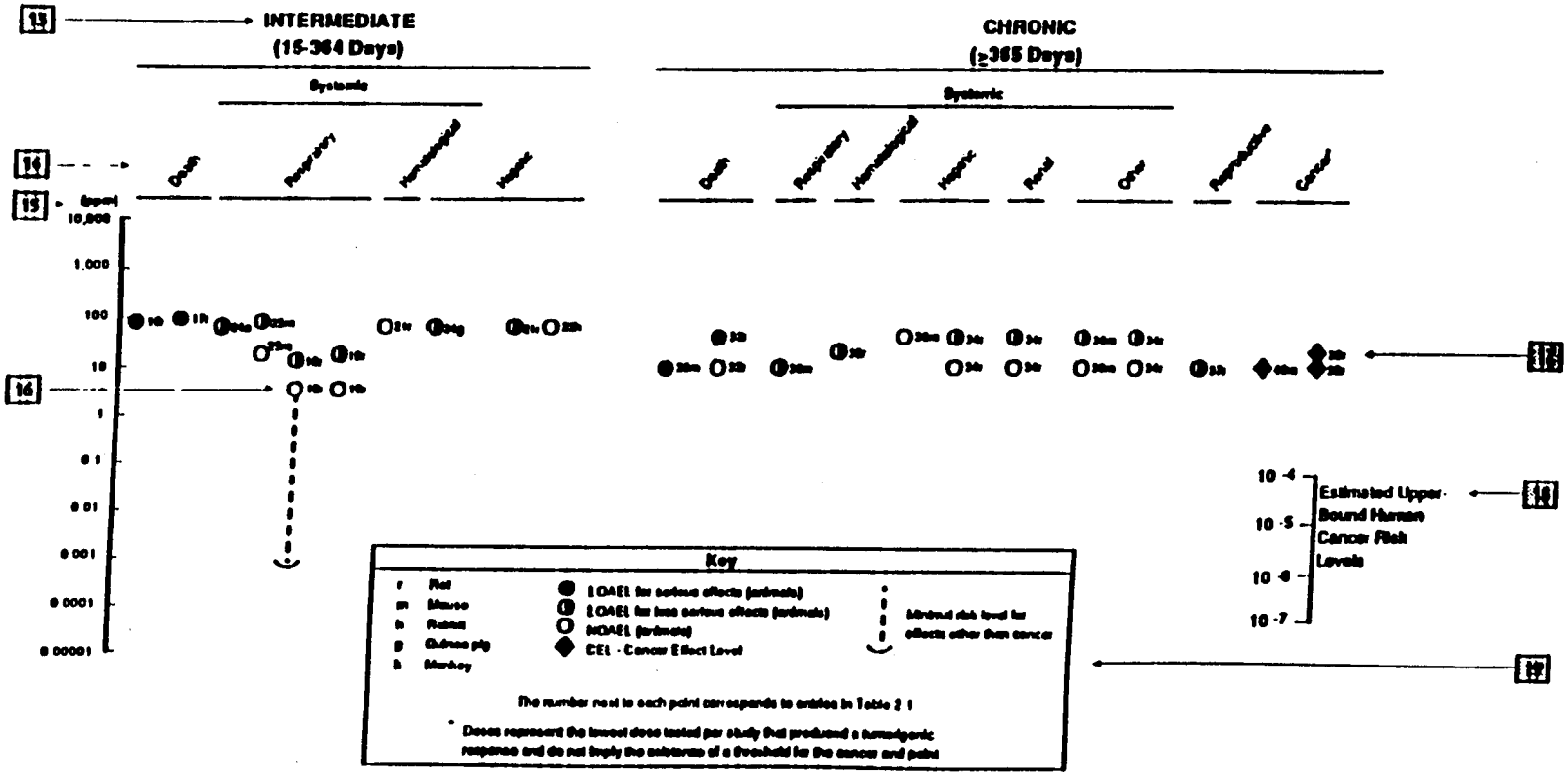

FIGURE 2-1. Levels of Significant Exposure to [Chemical X]-Inhalation

APPENDIX A

Chapter 2 (Section 2.4)

Relevance to Public Health

The Relevance to Public Health section provides a health effects summary based on evaluations of existing toxicological, epidemiological, and toxicokinetic information. This summary is designed to present interpretive, weight-of-evidence discussions for human health end points by addressing the following questions.

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

The section discusses health effects by end point. Human data are presented first, then animal data. Both are organized by route of exposure (inhalation, oral, and dermal) and by duration (acute, intermediate, and chronic). In vitro data and data from parenteral routes (intramuscular, intravenous, subcutaneous, etc.) are also considered in this section. If data are located in the scientific literature, a table of genotoxicity information is included.

The carcinogenic potential of the profiled substance is qualitatively evaluated, when appropriate, using existingtoxicokinetic, genotoxic, andcarcinogenic data. ATSDR does not currently assess cancer potency or perform cancer risk assessments. MRLs for noncancer end points if derived, and the end points from which they were derived are indicated and discussed in the appropriate section(s).

Limitations to existing scientific literature that prevent a satisfactory evaluation of the relevance to public health are identified in the Identification of Data Needs section.

Interpretation of Minimal Risk Levels

Where sufficient toxicologic information was available, MRLs were derived. MRLs are specific for route (inhalation or oral) and duration (acute, intermediate, or chronic) of exposure. Ideally, MRLs can be derived from all six exposure scenarios (e.g., Inhalation - acute, -intermediate, -chronic; Oral - acute, -intermediate, - chronic). These MRLs are not meant to support regulatory action, but to aquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans. They should help physicians and public health officials determine the safety of a community living near a substance emission, given the concentration of a contaminant in air or the estimated daily dose received via food or water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

APPENDIX A

MRL users should be familiar with the toxicological information on which the number is based. Section 2.4, "Relevance to Public Health," contains basic information known about the substance. Other sections such as 2.6, "Interactions with Other Chemicals" and 2.7, "Populations that are Unusually Susceptible" provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology used by the Environmental Protection Agency (EPA) (Barnes and Dourson, 1988; EPA 1989a) to derive reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the end point which, in its best judgement, represents the most sensitive humanhealth effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential effects (e.g., systemic, neurological, and developmental). In order to compare NOAELs and LOAELs for specific end points, all inhalation exposure levels are adjusted for 24hr exposures and all intermittent exposures for inhalation and oral routes of intermediate and chronic duration are adjusted for continous exposure (i.e., 7 days/week). If the information and reliable quantitative data on the chosen end point are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest NOAEL that does not exceed any adverse effect levels. The NOAEL is the most suitable end point for deriving an MRL. When a NOAEL is not available, a Less Serious LOAEL can be used to derive an MRL, and an uncertainty factor (UF) of 10 is employed. MRLs are not derived from Serious LOAELs. Additional uncertainty factors of 10 each are used for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans), In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the adjusted inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a substance-specific MRL are provided in the footnotes of the LSE Tables.

APPENDIX B

ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
ADME	Absorption, Distribution, Metabolism, and Excretion
ATSDR	Agency for Toxic Substances and Disease Registry
BCF	bioconcentration factor
BSC	Board of Scientific Counselors
CDC	Centers for Disease Control
CEL	Cancer Effect Level
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
CLP	Contract Laboratory Program
cm	centimeter
CNS	central nervous system
DHEW	Department of Health, Education, and Welfare
DHHS	Department of Health and Human Services
DOL	Department of Labor
ECG	electrocardiogram
EEG	electroencephalogram
EPA	Environmental Protection Agency
EKG	see ECG
FAO	Food and Agricultural Organization of the United Nations
FEMA	Federal Emergency Management Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
f_1	first generation
fpm	feet per minute
ft	foot
FR	Federal Register
g	gram
GC	gas chromatography
HPLC	high performance liquid chromatography
hr	hour
IDLH	Immediately Dangerous to Life and Health
IARC	International Agency for Research on Cancer
ILO	International Labor Organization
in	inch
Kd	adsorption ratio
kg	kilogram
Koc	octanol-soil partition coefficient
Kow	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{Lo}	lethal concentration low
LC_{50}	lethal concentration 50 percent kill
LD_{Lo}	lethal dose low
LD_{50}	lethal dose 50 percent kill
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
m	meter

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mg	milligram
min	minute
mL	milliliter
mm	millimeters
mmol	millimole
mppcf	millions of particles per cubic foot
MRL	Minimal Risk Level
MS	mass spectroscopy
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NIOSHTIC	NIOSH's Computerized Information Retrieval System
nm	nanometer
ng	nanogram
NHANES	National Health and Nutrition Examination Survey
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NOES	National Occupational Exposure Survey
NOHS	National Occupational Hazard Survey
NPL	National Priorities List
NRC	National Research Council
NTIS	National Technical Information Service
NTP	National Toxicology Program
OSHA	Occupational Safety and Health Administration
PEL	permissible exposure limit
pg	picogram
pmol	picomole
PHS	Public Health Service
PMR	proportional mortality ratio
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
REL	recommended exposure limit
RfD	Reference Dose
RTECS	Registry of Toxic Effects of Chemical Substances
sec	second
SCE	sister chromatid exchange
SIC	Standard Industrial Classification
SMR	standard mortality ratio
STEL	short-term exposure limit
STORET	STORAGE and RETRIEVAL
TLV	threshold limit value
TSCA	Toxic Substances Control Act
TRI	Toxic Release Inventory
TWA	time-weighted average
U.S.	United States
UF	uncertainty factor
WHO	World Health Organization
>	greater than
≥	greater than or equal to

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=	equal to
<	less than
≤	less than or equal to
%	percent
α	alpha
β	beta
δ	delta
γ	gamma
μm	micron
μg	microgram

APPENDIX C

PEER REVIEW

A peer review panel was assembled for bromomethane. The panel consisted of the following members: Dr. Judith S. Bellin, Private Consultant, Washington, DC; Dr. Caroline Holsapple (formerly Caroline Kramer), Department of Pharmacology and Toxicology, Medical College of Virginia, Richmond, VA; Dr. Norman M. Trieff, Department of Preventive Medicine and Community Health, The University of Texas Medical Branch, Galveston, TX; and Dr. Nancy Reiches, Private Consultant, Columbus, Ohio. These experts collectively have knowledge of bromomethane's physical and chemical properties, toxicokinetics, key health end points, mechanisms of action, human and animal exposure, and quantification of risk to humans. All reviewers were selected in conformity with the conditions for peer review specified in the Comprehensive Environmental Response, Compensation, and Liability Act of 1986, Section 104.

Scientists from the Agency for Toxic Substances and Disease Registry (ATSDR) have reviewed the peer reviewers' comments and determined which comments will be included in the profile. A listing of the peer reviewers' comments not incorporated in the profile, with a brief explanation of the rationale for their exclusion, exists as part of the administrative record for this compound. A list of databases reviewed and a list of unpublished documents cited are also included in the administrative record.

The citation of the peer review panel should not be understood to imply its approval of the profile's final content. The responsibility for the content of this profile lies with the ATSDR.

TOXICOLOGICAL PROFILE FOR 2-BUTANONE

Agency for Toxic Substances and Disease Registry
U.S. Public Health Service

July 1992

DISCLAIMER

The use of company or product name(s) is for identification only and does not imply endorsement by the Agency for Toxic Substances and Disease Registry.

FOREWORD

The Superfund Amendments and Reauthorization Act (SARA) of 1986 (Public Law 99-499) extended and amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). This public law directed the Agency for Toxic Substances and Disease Registry (ATSDR) to prepare toxicological profiles for hazardous substances which are most commonly found at facilities on the CERCLA National Priorities List and which pose the most significant potential threat to human health, as determined by ATSDR and the Environmental Protection Agency (EPA). The lists of the 250 most significant hazardous substances were published in the Federal Register on April 17, 1987; on October 20, 1988; on October 26, 1989; and on October 17, 1990. A revised list of 275 substances was published on October 17, 1991.

Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the lists. Each profile must include the following content:

(A) An examination, summary, and interpretation of available toxicological information and epidemiological evaluations on the hazardous substance in order to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects.

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure which present a significant risk to human health of acute, subacute, and chronic health effects.

(C) Where appropriate, an identification of toxicological testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

This toxicological profile is prepared in accordance with guidelines developed by ATSDR and EPA. The original guidelines were published in the Federal Register on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile is intended to characterize succinctly the toxicological and adverse health effects information for the hazardous substance being described. Each profile identifies and reviews the key literature (that has been peer-reviewed) that describes a hazardous substance's toxicological properties. Other pertinent literature is also presented but described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

Foreword

Each toxicological profile begins with a public health statement, which describes in nontechnical language a substance's relevant toxicological properties. Following the public health statement is information concerning levels of significant human exposure and, where known, significant health effects. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to protection of public health will be identified by ATSDR, the National Toxicology Program (NTP) of the Public Health Service, and EPA. The focus of the profiles is on health and toxicological information; therefore, we have included this information in the beginning of the document.

The principal audiences for the toxicological profiles are health professionals at the federal, state, and local levels, interested private sector organizations and groups, and members of the public.

This profile reflects our assessment of all relevant toxicological testing and information that has been peer reviewed. It has been reviewed by scientists from ATSDR, the Centers for Disease Control, the NTP, and other federal agencies. It has also been reviewed by a panel of nongovernment peer reviewers. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

William L. Roper

William L. Roper, M.D., M.P.H.
Administrator
Agency for Toxic Substances and
Disease Registry

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1. PUBLIC HEALTH STATEMENT

This Statement was prepared to give you information about 2-butanone(methyl ethyl ketone) and to emphasize the human health effects that may result from exposure to it. The Environmental Protection Agency (EPA) has identified 1,177 sites on its National Priorities List (NPL). 2-butanone has been found at 137 of these sites. However, we do not know how many of the 1,177 NPL sites have been evaluated for 2-butanone. As EPA evaluates more sites, the number of sites at which 2-butanone is found may change. The information is important for you because 2-butanone may cause harmful health effects and because these sites are potential or actual sources of human exposure to 2-butanone.

When a chemical is released from a large area, such as an industrial plant, or from a container, such as a drum or bottle, it enters the environment as a chemical emission. This emission, which is also called a release, does not always lead to exposure. You are exposed to a chemical only when you come into contact with the chemical. You may be exposed to it in the environment by breathing, eating, or drinking substances containing the chemical or from skin contact with it.

If you are exposed to a hazardous substance such as 2-butanone, several factors will determine whether harmful health effects will occur and what the type and severity of those health effects will be. These factors include the dose (how much), the duration (how long), the route or pathway by which you are exposed (breathing, eating, drinking, or skin contact), the other chemicals to which you are exposed, and your individual characteristics such as age, sex, nutritional status, family traits, life style, and state of health.

1.1 WHAT IS 2-BUTANONE?

2-Butanone, also known as methyl ethyl ketone (MEK), is a colorless liquid with a sweet, but sharp odor. 2-Butanone is manufactured in large amounts for use in paints, glues, and other finishes because it rapidly evaporates and will dissolve many substances, It will quickly evaporate into the air. 2-Butanone is often found dissolved in water or as a gas in the air. 2-Butanone is also a natural product made by some trees and is found in some fruits and vegetables. The exhausts of cars and trucks release 2-butanone into the air. 2-Butanone is usually found in the air, water, and soil of landfills and hazardous waste sites.

In water, 2-butanone can be changed to a more simple chemical form by natural biological processes and will be broken down in about 2 weeks. It will not be deposited in the sediment of rivers or lakes, and it is not expected to concentrate in fish. In air, 2-butanone will break down under the influence of sunlight, although it does not react with sunlight directly. One-half of any given amount of 2-butanone in the air will break down in 1 day or less. It is not known if 2-butanone changes to a more simple form by

natural biological processes in soil, but it is expected to do so because similar substances are broken down by these processes. 2-Butanone will not stick to soil, and if it is spilled onto soil, it will travel through the soil into underground water sources. Some of the 2-butanone found in soil or water will also evaporate to the air.

You will find more information on the chemical properties of 2-butanone in Chapter 3. The uses of 2-butanone are given in Chapter 4. More information on how 2-butanone behaves in the environment is found in Chapter 5.

1.2 HOW MIGHT I BE EXPOSED TO 2-BUTANONE?

2-Butanone can enter the environment in a number of different ways. It can enter the air or water from the waste of manufacturing plants. 2-Butanone is present in many different types of paints and glues used both in the home and in industry. As these products dry, 2-butanone will enter the air. 2-Butanone is also in air because it is released in the exhaust of cars and trucks. Some trees in the forest release 2-butanone to the air.

We do not know the background levels of 2-butanone in air, water, or soil. We know that 2-butanone is found naturally in some foods. We know it is found at hazardous waste sites, and it is also found occasionally in drinking water and often in the air of cities. You may also be exposed to 2-butanone by smoking cigarettes.

You may be exposed to higher levels of 2-butanone if you use glues of coatings containing it in a small enclosed area that does not have good air flow, People who use it at work have a good chance of being exposed to 2-butanone. 2-butanone is used in such industries as shoes factories, printing plants, plastics factories, and sporting goods manufacturers. People who live near a toxic waste site where 2-butanone is kept may breathe it if it evaporates into the air, or drink it if it gets into the water supply, especially when the water supply comes from wells.

You can find more information on how much 2-butanone is in the environment and how you can be exposed to it in Chapter 5.

1.3 HOW CAN 2-BUTANONE ENTER AND LEAVE MY BODY?

2-butanone can enter your body if you breathe air that contains it, through your skin if it touches you, or through your mouth if you eat food or drink water that has 2-butanone in it. Studies have shown that, if there is 2-butanone in the air you breathe, at least half of what you breathe in will enter your body. The other half will leave in the air you breathe out. We do not know how much 2-butanone will stay in your body if you drink it or if it touches your skin. The amount of 2-butanone that actually enters your body depends on how much is in the air you breathe, how much is in your food or

water, or how much gets on your skin. The amount of 2-butanone that enters your body also depends on how long you breathe it or how long it is on your skin before you wash it off. Your body gets rid of 2-butanone in urine and in the air you breathe out. 2-Butanone is not a chemical that stays in your body for very long; it will be gone by the next day. For more information on how 2-butanone gets into and leaves your body, see Chapter 2.

1.4 HOW CAN 2-BUTANONE AFFECT MY HEALTH?

Some people who breathed air that contained 2-butanone first noticed its sweet, sharp odor at a concentration of 5-8 parts of 2-butanone per million parts of air (5-8 ppm). The main health effects that have been seen in humans who breathed higher concentrations of 2-butanone are mild irritation of the nose, throat, eyes, and skin.

Serious health effects in animals have been seen only at very high concentrations of 2-butanone. These high concentrations are not expected in the usual use of 2-butanone or in the vicinity of hazardous waste sites. Studies in animals have shown that 2-butanone does not cause serious damage to the nervous system or the liver, but mice that breathed low levels for a short time had temporary behavioral effects. 2-Butanone alone does not have serious effects on the liver or nervous system, but it can cause other chemicals to become more harmful to these systems.

Guinea pigs, rats, and mice that breathed high levels of 2-butanone for a short time became unconscious and died. Pregnant rats and mice that breathed air containing high levels of 2-butanone had underdeveloped fetuses. The rats that swallowed very high concentrations of 2-butanone in water also developed signs of nervous system effects such as inactivity, drooping eye lids, and uncoordinated muscle movement. Some rats and mice that swallowed water containing high concentrations of 2-butanone died. Rats that received water containing a lower concentration of 2-butanone had mild kidney damage. Skin irritation developed in rabbits and guinea pigs that had small amounts of 2-butanone dropped on their skin. Rabbits that had small amounts of 2-butanone dropped in their eyes had serious eye irritation. We do not know whether 2-butanone causes birth defects or affects reproduction in humans. Reproductive effects were not seen in animals exposed to 2-butanone. We have no information about whether 2-butanone causes cancer in humans or animals.

A more complete discussion of the health effects of 2-butanone in humans and animals can be found in Chapter 2.

1.5 IS THERE A MEDICAL TEST TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO 2-BUTANONE?

No specific medical test is available to determine whether you have been exposed to 2-butanone. Studies in humans and animals have shown that it is possible to detect 2-butanone or its breakdown products in the blood, breath,

and urine. The levels of 2-butanone found in the blood, breath, and urine are usually associated with the levels of exposure found in the workplace, but this is more useful for determining exposure of groups of people rather than individuals. Tests for 2-butanone in blood, urine, or breath are useful only for recent exposure because 2-butanone and its breakdown products leave the body rapidly. These considerations are discussed in more detail in Chapters 2 and 6.

1.6 WHAT RECOMMENDATIONS HAS THE FEDERAL GOVERNMENT MADE TO PROTECT HUMAN HEALTH?

The Occupational Safety and Health Administration (OSHA) has set an occupational exposure limit of 200 ppm of 2-butanone in the air. The National Institute for Occupational Safety and Health (NIOSH) has also recommended 200 ppm of 2-butanone as the limit for up to a 10-hour work shift in a 40-hour workweek. Because of its odor, you can smell 2-butanone before it harms you. Further information on governmental recommendations can be found in Chapter 7.

1.7 WHERE CAN I GET MORE INFORMATION?

If you have any more questions or concerns not covered here, please contact your state health or environmental department or:

Agency for Toxic Substances and Disease Registry
Division of Toxicology
1600 Clifton Road, E-29
Atlanta, Georgia 30333

This agency can also provide you with information on the location of the nearest occupational and environmental health clinic. Such clinics specialize in recognizing, evaluating, and treating illnesses that result from exposure to hazardous substances.

2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective of the toxicology of 2-butanone (methyl ethyl ketone) and a depiction of significant exposure levels associated with various adverse health effects. It contains descriptions and evaluations of studies and presents levels of significant exposure for 2-butanone based on toxicological studies and epidemiological investigations.

2-Butanone alone is a relatively safe chemical widely used as a insolvent industry. For some uses, 2-butanone is combined with other chemicals thathave serious neurotoxic and hepatotoxic effects. Clinical reports and animal studies have clearly shown that exposure to 2-butanone alone causes minimal chronic neurological or hepatic deficits, if any. It does potentiate both the neurotoxicity of n-hexane and methyl-n-butyl ketone and the hepatotoxicity of carbon tetrachloride and chloroform. The potentiation of neurotoxicity and hepatotoxicity by 2-butanone is discussed in Section 2.6 (Interactions with other Chemicals).

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure--inhalation, oral, and dermal--and then by health effect--death, systemic, immunological, neurological, developmental, reproductive, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods--acute (less than 15 days), intermediate (15-364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELS) or lowest-observed-adverse-effect levels (LOAELS) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. These distinctions are intended to help the users of the document identify the levels of exposure at which adverse health effects start to appear. They should also help to determine whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the tables and figures may differ depending on the user's perspective. For example, physicians concerned with the interpretation of clinical findings in exposed persons may be interested in levels of exposure associated with "serious" effects. Public health officials and project managers concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAEL) or exposure

levels below which no adverse effects (NOAEL) have been observed. Estimates of levels posing minimal risk to humans (minimal risk levels, MRLs) may be of interest to health professionals and citizens alike.

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made, where data were believed reliable, for the most sensitive noncancer effect for each exposure duration. MRLs include adjustments to reflect human variability from laboratory animal data to humans.

Although methods have been established to derive these levels (Barnes et al. 1988; EPA 1989a), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

2.2.1 Inhalation Exposure

2.2.1.1 Death

No studies were located regarding death of humans following inhalation exposure to 2-butanone.

Acute (4-hour) exposure to 2,000 ppm 2-butanone caused the death of up to 4 of 6 rats within a 14-day observation period after exposure (Carpenter et al. 1949). The cause of death was not reported, but gross necropsy and histopathology confirmed that extraneous infections were not involved. In contrast, exposure of 6 rats to 8,000 ppm 2-butanone for 8 hours resulted in the death of half of the rats (Smyth et al. 1962). Furthermore, the 4-hour LC_{50} in rats, calculated from the dose-response curve, was 11,700 ppm (LaBelle and Brieger 1955). The LC_{50} was determined using similar rats and the same exposure methods used by Carpenter et al. (1949). Mice exposed to a saturated vapor of 2-butanone (estimated concentration: 103,000 ppm) showed a mean survival time of 43 minutes (LaBelle and Brieger 1955). The LT_{50} and LT_{50} in rats exposed to 92,239 ppm 2-butanone were 3 and 0.5 hours, respectively (Klimisch 1988). The LT_{50} represents the time of exposure after which 50% of the animals died within 14 days following exposure. The LT_0 represents the time of exposure after which no animals died within 14 days following exposure. No deaths were reported after a 4-hour exposure of mice to 2,438 ppm 2-butanone (De Ceaurriz et al. 1983). Death of guinea pigs occurred within 45 minutes of exposure to 100,000 ppm 2-butanone and within 200 minutes of exposure to 33,000 ppm (Patty et al. 1935). Gasping respiration was observed at concentrations of 33,000 ppm and higher about 10 minutes before the guinea pigs died.

In intermediate duration studies, no deaths occurred during a 90-day exposure of rats to 5,000 ppm or less, 5 days/week for 6 hours/day (Cavenderet al. 1983). In contrast, five of five rats died within 7 weeks of a planned 15-week exposure to 6,000 ppm, 7 days/week, 8 hours/day (Altenkirch et al. 1978a). The cause of death for all rats exposed to 2-butanone in this study was severe bronchopneumonia confirmed pathologically and histologically. In the same study, rats exposed to n-hexane or a combination of n hexane and 2-butanone did not develop bronchopneumonia. A repeat of this study gave the same results, i.e., death within 7 weeks coincident with confirmed bronchopneumonia (Altenkirch et al. 1978b). Saida et al. (1976) reported no deaths or change in clinical signs in rats exposed to 1,125 ppm 2-butanone continuously for 5 months. Similar results were reported by several groups after intermediate exposures ranging from 200 to 800 ppm in rats and guinea pigs, i.e., no deaths and no change in clinical signs (LaBelle and Brieger 1955; Takeuchi et al. 1983; Toftgard et al. 1981). The LC_{50} the highest NOAEL values, and all reliable LOAEL values for death in each species and duration category are recorded in Table 2-l and plotted in Figure 2-l.

2.2.1.2 Systemic Effects

The systemic effects of 2-butanone following inhalation exposure are discussed below. No studies were located regarding cardiovascular, gastrointestinal, musculoskeletal, hepatic, or renal effects in humans after inhalation exposure to 2-butanone. The highest NOAEL values and all reliable LOAEL values for each systemic effect for each species and duration category are recorded in Table 2-l and plotted in Figure 2-l.

Respiratory Effects. 2-Butanone is irritating to respiratory tissues. A clinical case report of three men exposed to 2-butanone fumes while removing paint from an airplane hangar noted mild respiratory symptoms but did not further describe the nature or extent of the symptoms (Berg 1971). Volunteers exposed to 100 ppm 2-butanone complained of slight nose and throat irritation, which became objectionable at 300 ppm (Nelson et al. 1943). The respiratory tract irritation noted in humans at 100 ppm does not imply that humans are more sensitive to the respiratory effects of 2-butanone than other species tested (see Table 2-l). Another possible explanation is that humans *are* better able to communicate the early signs of irritation compared with the other species tested. Nasal resistance was significantly increased in humans upon exposure to the threshold level of 2-butanone; this response reflects a nasopharyngeal reflex (Doty et al. 1988). The odor threshold for 2-butanone falls in the range 5.4-8.25 ppm (Amoore and Hautala 1983; Doty et al. 1988).

At high concentrations, 2-butanone is also irritating to respiratory tissues of animals. Severe upper respiratory tract irritation was found after a few days in rats exposed to 10,000 ppm, 8 hours/day (Altenkirch et al. 1978a). Guinea pigs exposed to 33,000 ppm had gasping respiration after 180 minutes of exposure and died after 200-260 minutes of exposure. Their

TABLE 2-1. Levels of Significant Exposure to 2-Butanone - Inhalation

Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
ACUTE EXPOSURE							
Death							
1	Rat	1 d 4 hr/d				11,700 (LC_{50})	LaBelle and Brieger 1955
2	Rat	1 d 8 hr/d				8,000 (3/6 died)	Smyth et al. 1962
3	Rat	1 d 3 hr/d				92,239	Klimisch 1988
4	Gn pig	1 d 3 hr/d		10,000		33,000	Patty et al. 1935
5	Mouse	43 min				103,000	LaBelle and Brieger 1955
Systemic							
6	Human	1 d 5 min/d	Resp		100 (nose/throat irritation)		Nelson et al. 1943
7	Rat	a few d 8 hr/d	Resp		10,000 (respiratory irritation)		Altenkirch et al. 1978a
8	Rat	7 d 8 hr/d	Hepatic	300			Li et al. 1986
9	Gn pig	1 d 13.5 hr/d	Resp Hepatic Renal Derm/oc	10,000 3,300 3,300 3,300	 10,000 (congestion) 10,000 (congestion) 10,000 (eye irritation, lacrimation)	33,000 (gasping and death) 100,000 (corneal opacity and death)	Patty et al. 1935
Neurological							
10	Human	1 d 4 hr/d		200			Dick et al. 1984, 1988, 1989
11	Gn pig	1 d 13.5 hr/d		3,300		10,000 (narcosis, incoordination)	Patty et al. 1935
12	Mouse	1 d 4 hr/d			1,602 (reduced immobility)		De Ceaurriz et al. 1983

TABLE 2-1 (Continued)

Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
Developmental							
13	Rat	10 d Gd 6-15 7 hr/d		1,000		3,000 (extra ribs, delayed ossification)	Deacon et al. 1981
14	Mouse	10 d Gd 6-15 7 hr/d		1,000		3,000 (decreased fetal body weight, sternebral anomalies)	Mast et al. 1989
INTERMEDIATE EXPOSURE							
Death							
15	Rat	12 wk 5 d/wk 7 hr/d		235			LaBelle and Brieger 1955
16	Rat	90 d 5 d/wk 6 hr/d		5,000			Cavender et al. 1983
17	Rat	7 wk 7 d/wk 8 hr/d				6,000 (5/5 died)	Altenkirch et al. 1978a, 1978b
18	Gn pig	12 wk 5 d/wk 7 hr/d		235			LaBelle and Brieger 1955
Systemic							
19	Rat	90 d 5 d/wk 6 hr/d	Resp Cardio Gastro Hemato Musc/sk Hepatic Renal Derm/oc Other	5,000 5,000 5,000 5,000 5,000 5,000 5,000 5,000 5,000			Cavender and Casey 1981; Cavender et al. 1983
Immunological							
20	Rat	90 d 5 d/wk 6 hr/d		5,000			Cavender and Casey 1981; Cavender et al. 1983

TABLE 2-1 (Continued)

Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
Neurological							
21	Rat	7 wk 7 d/wk 8 hr/d		6,000			Altenkirch et al. 1978a, 1978b
22	Rat	90 d 5 d/wk 6 hr/d		5,000			Cavender and Casey 1981; Cavender et al. 1983
Reproductive							
23	Rat	90 d 5 d/wk 6 hr/d		5,000			Cavender and Casey 1981; Cavender et al. 1983

[a]The number corresponds to entries in Figure 2-1.

Cardio = cardiovascular; d = day; Derm/oc = dermal/ocular; Gastro = gastrointestinal; Gd = gestational day; Gn pig = guinea pig; Hemato = hematological; hr = hour(s); LC_{50} = lethal concentration 50% kill; LOAEL = lowest-observed-adverse-effect level; min = minutes; Musc/sk = musculoskeletal; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s)

FIGURE 2-1. Levels of Significant Exposure to 2-Butanone - Inhalation

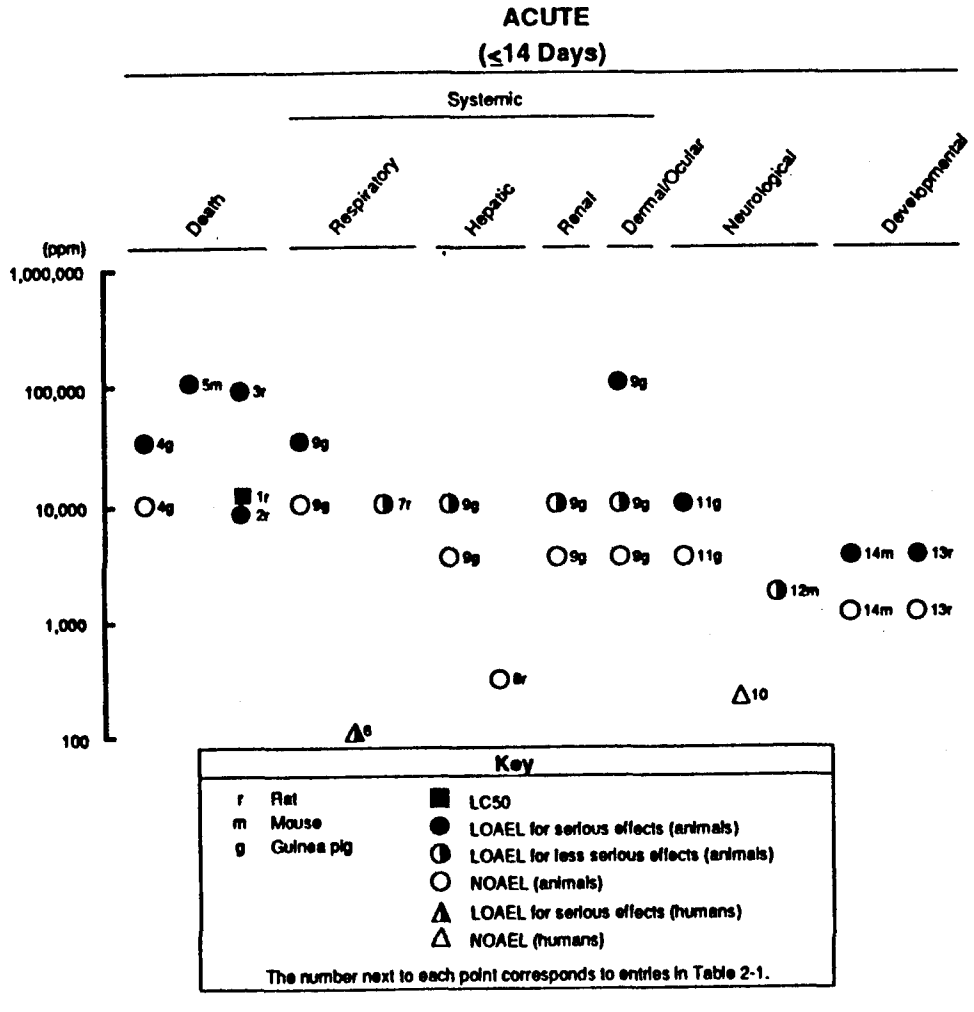
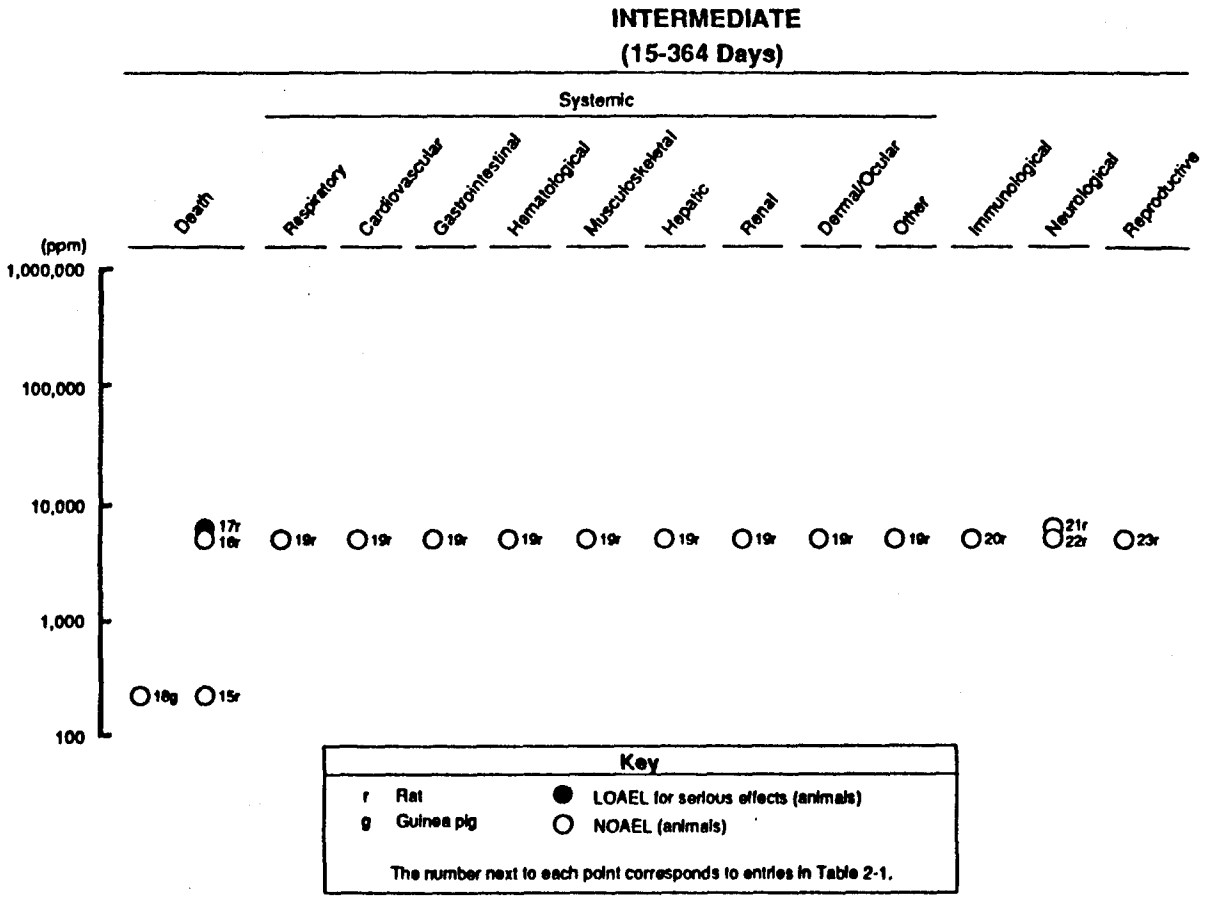


FIGURE 2-1 (Continued)



lungs were emphysematous. Rats seem to tolerate concentrations that are still high, but substantially lower than the acute exposures when exposed intermittently in intermediate duration studies. In a 90-day inhalation study, exposure of rats to 2-butanone concentrations of 5,000 ppm or less caused no signs of upper respiratory tract irritation or other respiratory effects (Cavender et al. 1983). Due to the irritation observed at 10,000 ppm in the study by Altenkirch et al. (1978a), the exposure concentration was reduced to 6,000 ppm and the study continued. All the rats died suddenly at 7 weeks with pathologically confirmed bronchopneumonia. This experiment was repeated and had the same results (Altenkirch et al. 1978b). Furthermore, rats exposed to n-hexane or a combination of n-hexane and 2-butanone did not develop bronchopneumonia, suggesting that a factor other than poor animal maintenance precipitated the bronchopneumonia. The Wistar rats used in this study may possibly have been derived from a stock that was particularly susceptible to infection. The initial exposure to a high concentration of 2-butanone may have weakened their immune system allowing infection to develop. No other studies were located that reported a link between 2-butanone exposure and bronchopneumonia in humans or animals.

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after inhalation exposure to 2-butanone.

Histological examination of the hearts and aortae of rats exposed to 5,000 ppm or less of 2-butanone for 90 days revealed no exposure-related lesions (Cavender and Casey 1981; Cavender et al. 1983).

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans after inhalation exposure to 2-butanone.

No histopathological lesions were found in the esophagus, salivary glands, ileum, duodenum, jejunum, cecum, large or small intestines, or pancreas of rats exposed to 5,000 ppm or less of 2-butanone for 90 days(Cavender and Casey 1981; Cavender et al.1983).

Hematological Effects. Information regarding hematological effects of 2-butanone exposure in humans is limited to a case report in which a normal hematological profile and blood chemistry were found in an 18-year-old seaman exposed to 2-butanone while removing paint from an airplane hangar (Berg 1971). 2-Butanone exposure in this case was linked to retrobulbar neuritis and severely impaired vision. However, because methanol was found in the blood of the patient, consumption or exposure to methanol cannot be ruled out.

Studies in animals also indicate that 2-butanone does not produce hematological effects. No effect on hemoglobin concentration, or on red blood cell, white blood cell, neutrophil, lymphocyte, or monocyte populations were observed in rats exposed intermittently to 235 ppm 2-butanone for 12 weeks(LaBelle and Brieger 1955). Similarly, the hematological profile and serum

chemistry of rats exposed to 5,000 ppm or less of 2-butanone for 90 days were normal (Cavender et al. 1983).

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans after inhalation exposure to 2-butanone.

Histological examination of skeletal muscle and bone of rats exposed to 5,000 ppm or less of 2-butanone for 90 days revealed no exposure-related lesions (Cavender and Casey 1981; Cavender et al. 1983).

Hepatic Effects. No studies were located regarding hepatic effects in humans after inhalation exposure to 2-butanone.

Most of the hepatic effects of inhalation exposure to 2-butanone observed in animals are minimal and probably not adverse, although acute exposure of guinea pigs to a high concentration (10,000 ppm) caused liver congestion (Patty et al. 1935). Exposure to 3,300 ppm had no effects. Serum alkaline phosphatase activity was not different in rats exposed intermittently to 300 ppm 2-butanone for 7 days compared to nonexposed control rats (Li et al. 1986). There was no change in the isozymes of cytochrome P-450 or in the total concentration of cytochrome P-450 in rats exposed to 800 ppm for 4 weeks(Toftgard et al. 1981). 2-Butanone, however, altered the metabolism of androstenedione by increasing the formation of two metabolites and decreasing the formation of two other metabolites. Furthermore, liver weight was increased in the 2-butanone-exposed rats (Toftgard et al. 1981). A small but statistically significant increase in absolute and relative liver weights of male and female rats, but no change in serum levels of hepatic enzymes (serum glutamic-oxaloacetic transaminase [SGOT], serum glutamic-pyruvic transaminase[SGPT], serum gamma-glutamyl transpeptidase [SGGT], and alkaline phosphatase)in male rats, was observed at an exposure level of 5,000 ppm for 90 days (Cavender et al. 1983). A significant increase only in alkaline phosphatase was noted in the female rats. Histopathological examination did not reveal any hepatic lesion aside from those expected in Fischer rats of this age. Exposure to 2,500 ppm 2-butanone had no effect on any hepatic parameter (Cavender et al. 1983). In the absence of histopathological liver lesions, the mild liver effects observed at 5,000 ppm were probably not adverse.

Renal Effects. No studies were located regarding renal effects in humans following inhalation exposure to 2-butanone.

Acute inhalation exposure of guinea pigs to 10,000 ppm 2-butanone resulted in congestion of the kidney (Patty et al. 1935). No effects were observed at 3,300 ppm. In an intermediate duration study, only minimal kidney effects were observed in rats exposed to 5,000 ppm or less (Cavender et al. 1983). Blood urea nitrogen determinations and urinalysis including urine volume, specific gravity, and pH showed that all values were within normal limits for male and female rats; the exception was that urine volume in the females was slightly but significantly increased. The kidney/body weight

ratio in male rats and the kidney/brain weight ratio in female rats were slightly but significantly elevated. Histopathological examination did not reveal any treatment-related renal lesion. In the absence of histopathological lesions or decrements in kidney function, these mild kidney effects do not appear to be adverse. No other studies were located regarding the renal effects of inhalation exposure to 2-butanone.

Dermal/Ocular Effects. Two men exposed to 2-butanone while removing paint from an airplane hangar had conjunctival irritation (Berg 1971). A third man had severe loss of vision. Within 36 hours, the man's vision was completely restored. However, because methanol was found in the blood of the man with vision loss, exposure to methanol cannot be ruled out. No other studies were located regarding dermal/ocular effects in humans following inhalation exposure to 2-butanone.

Guinea pigs exposed to 2-butanone. concentrations of 10,000 ppm or greater had eye irritation and lacrimation (Patty et al. 1935). Exposure to 100,000 ppm for 30 minutes or more caused cornea1 opacity. This condition gradually improved in guinea pigs that lived to 8 days after exposure. No effects occurred when guinea pigs were exposed to 3,300 ppm. Ophthalmological examination of the eyes and histological examination of the skin revealed no effects in rats exposed to 5,000 ppm or less of 2-butanone for 90 days (Cavender and Casey 1981; Cavender et al. 1983). No other studieswere located regarding dermal/ocular effects in animals following inhalation exposure to 2-butanone.

Other Systemic Effects. No studies were located regarding other systemic effects in humans after inhalation exposure to 2 butanone.

In rats, no histopathological lesions were found in the thyroid, parathyroid, pituitary gland, adrenal glands, ears, or Zymbal glands of rats exposed to 5,000 ppm or less of 2-butanone for 90 days (Cavender and Casey 1981; Cavender et al. 1983). Furthermore, no specific effects on body weight were found.

2.2.1.3 Immunological Effects

No studies were located regarding immunological effects in humans following inhalation exposure to 2-butanone.

Although no specific tests for immunological effects were performed, histological examination of lymph nodes, thymus, spleen, and bone marrow of rats exposed to 5,000 ppm or less of 2-butanone for 90 days revealed no exposure-related lesions (Cavender and Casey 1981; Cavender et al. 1983). This NOAEL value is recorded in Table 2-l and plotted in Figure 2-l.

2.2.1.4 Neurological Effects

In three separate studies, volunteers underwent a single 4-hour exposure to 200 ppm 2-butanone (Dick et al. 1984, 1988, 1989). No differences were observed between exposed and control groups on neurobehavioral tests including psychomotor tests (choice reaction time, visual vigilance, dual task, and memory scanning), postural sway, and a profile of mood states. No other studies were located regarding neurological effects after inhalation exposure to 2-butanone.

Neurological effects have been observed in animals exposed by inhalation to 2-butanone. Exposure of mice to 2-butanone at concentrations greater than or equal to 1,602 ppm for 4 hours caused a dose-related reduction in the duration of immobility in a "behavioral despair" swimming test (De Ceaurriz et al. 1983). The authors noted that the effect of 2-butanone was similar to that of antidepressants. In guinea pigs exposed acutely to 10,000 ppm 2-butanone, incoordination occurred within 90 minutes and unconsciousness occurred within 240-280 minutes (Patty et al. 1935). These signs occurred earlier at higher concentrations, but no neurological signs were observed at 3,300 ppm. Juvenile baboons exposed continuously to 100 ppm for 7 days showed early signs of narcosis, incoordination, and a loss of time perception in neurobehavioral tests (Geller et al. 1979). The neurological effects observed in this study could have resulted from narcosis. It is also possible that the baboons were distracted during the testing due to the irritating effects of 2-butanone on the respiratory system. Furthermore, the effects of 2-butanone observed at 100 ppm in the baboons do not imply that baboons are more sensitive to 2-butanone than other species tested. Since the baboons were evaluated with a complex discriminant behavioral task, it is possible that subtle neurobehavioral effects could be observed. However, it should be noted that only one exposure level was tested, only one baboon of four tested showed consistently different results from the controls throughout the study, and no statistical tests were performed. These limitations preclude definitive conclusions.

Intermediate duration exposures to 2-butanone were not neurotoxic in rats. Male Sprague-Dawley rats exposed continuously to 1,125 ppm 2-butanone for periods of 5 months or less showed no signs of peripheral neuropathy following histological examination (Saida et al. 1976). The neurotoxicity of n-butyl ketone, however, was markedly potentiated by 2-butanone. No differences were observed in nerve fiber preparations from male and female Fischer 344 rats exposed to 5,000 ppm or less 2-butanone for 90 days (Cavender and Casey 1981; Cavender et al. 1983). Furthermore, no histopathological lesions were found in the brain, sciatic nerve, tibia1 nerve, spinal cord, or optic nerves. No effects were observed in posture, gait, tone, and symmetry of the facial muscles, or in the pupillary, palpebral, extensor thrust, and cross-extensor thrust reflexes. The only effect recorded was a slight but statistically significant increase in brain weight in female rats exposed to 5,000 ppm. No clinical signs and no histological evidence of neuropathy in

peripheral nerves from the brachial plexus, sciatic nerve, spinal cord, and medulla were observed in rats exposed to 6,000 ppm for 7 weeks compared with rats exposed to n-hexane or a combination of n-hexane and 2-butanone(Altenkirch et al. 1978a). In contrast, 2-butanone potentiated the neurotoxicity of n-hexane. No neuropathological changes were found on light microscope and electron microscope examination of teased tail nerves after exposure of a rat to 200 ppm 2-butanone for 24 weeks (Takeuchi et al. 1983). At 4 weeks, significant increases in motor nerve conduction velocity and mixed nerve conduction velocity were found, while distal motor latency was decreased. These changes in nerve conduction velocity were not seen beyond 4 weeks. The transient increase in nerve conduction velocity may have been due to an effect of 2-butanone on the axonal membrane (Takeuchi et al. 1983). The highest NOAEL values and all reliable LOAEL values for neurological effects in each species and duration category are recorded in Table 2-l and plotted in Figure 2-l.

2.2.1.5 Developmental Effects

No studies were located regarding developmental effects in humans after inhalation exposure to 2-butanone.

Several studies in rats and mice were located regarding developmental effects after inhalation exposure. Exposure of pregnant rats to 1,000 or 3,000 ppm 2-butanone during gestation resulted in a slight increase in the incidence of malformations at 3,000 ppm; acaudia and imperforate anus were found in 2 fetuses out of 21 litters, and brachygnathia was noted in 2 other fetuses (Schwetz et al. 1974). A low incidence of sternebral anomalies was also noted in the 3,000 ppm group. Although the incidence of malformations was not high enough to support a positive correlation, it may have indicated a slight teratogenic effect in rats. A second study by the same group supported the previous findings of skeletal anomalies (Deacon et al. 1981). No statistically significant differences in external or soft tissue abnormalities were found in the offspring of dams exposed to 3,000 ppm or less during gestation, No effect was observed on the number of live fetuses/litter or on fetal crown-rump length. Skeletal abnormalities, including delayed ossification of the cervical centra, sternebral malformations, and asymmetric pelvis were observed at 3,000 ppm. Decreased body weight gain and increased water consumption in the pregnant rats at 3,000 ppm 2-butanone indicated that some maternal toxicity may have occurred at this exposure level. Deacon et al. (1981) concluded 2-butanone was slightly fetotoxic, but not embryotoxic or teratogenic at 3,000 ppm. Mean fetal body weight was reduced in the male and female offspring of mouse dams exposed to 3,000 ppm butanone, but was significantly reduced only in the males(Mast et al. 1989). A statistically significant increase in the incidence of misaligned sternebrae was observed in the 3,000 ppm group. No effects were observed at 1,000 ppm. Thus, 2-butanone was fetotoxic in both rats and mice. In pregnant rats, continuous exposure to 800 ppm 2-butanone throughout gestation resulted in the failure of three of eight of the rats to deliver

litters. While all 8 of the control dams in the experiment for 2-butanone delivered litters, 6 of 16 control dams in an experiment with n-hexane in the same study also failed to produce litters. Therefore, the reliability of the results in the 2-butanone exposed group is questionable (Stoltenburg-Didinger et al. 1990). The reliable NOAEL and LOAEL values for developmental effects are recorded in Table 2-l and plotted in Figure 2-l.

2.2.1.6 Reproductive Effects

No studies were located regarding reproductive effects in humans after inhalation exposure to 2-butanone.

Although no tests for reproductive function were performed, histological examination of the testes, epididymides, seminal vesicles, vaginas, cervices, uteri, oviducts, ovaries, or mammary glands of rats exposed to 5,000 ppm or less of 2-butanone for 90 days revealed no exposure-related lesions (Cavender and Casey 1981; Cavender et al. 1983).

2.2.1.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or animals after inhalation exposure to 2-butanone.

Genotoxicity studies are discussed in Section 2.4.

2.2.1.8 Cancer

Two retrospective studies of industrial workers chronically exposed to 2-butanone in dewaxing plants reported that deaths due to cancer were less than expected. In a cohort of 446 males employed by Shell Chemical Company, 13 deaths were due to cancer, whereas 14.26 were expected; the standard mortality ratio (SMR) was 0.91 (Alderson and Rattan 1980). In the same cohort, 2 cases of buccal or pharyngeal neoplasms were found; 0.13 were expected to exist, and the' SMR was 15.38. There were 4 cases of stomach, colon, or rectal cancer; 3.18 were expected, and the SMR was 1.28. The incidence of buccal or pharyngeal neoplasms was statistically significant but was regarded by the authors as due to chance because of the small number of individuals affected and the number of separate comparisons made between observed and expected rates. Furthermore, the use of tobacco was not discussed in this study. The incidence of stomach, colon, or rectal cancer was not statistically significant. The authors concluded that there was no clear evidence of a cancer hazard at this dewaxing plant. A retrospective cohort study of 1,008 male oil refinery workers occupationally exposed to an estimated l-4 ppm of 2-butanone in a dewaxing-lubricating oil plant was also conducted (Wen et al. 1985). The overall cancer-related mortality was less than expected. The increased incidence of buccal and pharyngeal neoplasms reported by Alderson and Rattan (1980) was not confirmed in this study.

No studies were located regarding cancer in animals following inhalation exposure to 2-butanone.

2.2.2 Oral Exposure

2.2.2.1 Death

No studies were located regarding death of humans following oral exposure to 2-butanone.

Oral LD_{50} values for 2-butanone were similar (approximately 2,737 mg/kg) in three groups of Sprague-Dawley rats: immature (14 days old), young adult(80-160 g), and older adult (300-470 g) (Kimura et al. 1971). The oral LD_{50} could not be determined in newborn rats because of volume limitations; it was estimated to be less than 805 mg/kg, Most of the Sprague-Dawley rats receiving 3,670, 7,340, or 14,680 mg/kg by gavage died within 1 hour at each dose, except 1 male and 1 female at the lowest dose; these rats survived until sacrifice at 14 days (Stillmeadow Inc. 1978). The data were insufficient for determination of an LD_{50}, but the authors estimated the acute oral LD_{50} to be less than 3,670 mg/kg, which is in agreement with the data reported in Kimura et al. (1971). The LD_{50} in Carworth-Wistar rats was 5,522 mg/kg (Smyth et al. 1962), which may represent a strain difference. In two separate experiments, 1,080 mg 2-butanone/kg administered by gavage in corn oil produced no deaths in male Fischer rats (Brown and Hewitt 1984) or in male Sprague-Dawley rats(Hewitt et al. 1983). Tanii et al. (1986) determined the oral LD_{50} for 2-butanone in mice as 4,044 mg/kg (95% confidence limits - 3,200-5,111 mg/kg). The acute duration LD_{50} values and the LOAEL value for death in rats are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.2 Systemic Effects

The systemic effects of 2-butanone after oral exposure are discussed below. No studies were located regarding gastrointestinal, hematological, musculoskeletal, or dermal/ocular effects in humans or animals after oral exposure to 2-butanone. The highest NOAEL values and all reliable LOAEL values for each systemic effect after oral exposure in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

Respiratory Effects. One clinical report of oral exposure to 2-butanone in humans was located. A 47-year-old woman accidentally ingested an unknown volume of 2-butanone that had been stored in a rum bottle (Kopelman and Kalfayan 1983). She was admitted to an emergency ward unconscious and hyperventilating. Blood gases were 85 mmHg oxygen and 24 mmHg carbon dioxide. Analysis of her blood showed a 2-butanone plasma concentration of 95 mg/lOO mL. Slow infusion of sodium bicarbonate reduced the hyperventilation, and blood gases improved to 78 mmHg oxygen and 25 mmHg carbon dioxide. Within 12 hours, she had regained consciousness, made an

TABLE 2-2. Levels of Significant Exposure to 2-Butanone - Oral

Key to figure[a]	Species	Route	Exposure frequency/ duration	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
ACUTE EXPOSURE								
Death								
1	Rat	(G)	1 d				5,522 (LD_{50})	Smyth et al. 1962
2	Rat	(G)	1 d				3,670 (8/10 died)	Stillmeadow Inc. 1978
3	Rat	(G)	1 d				2,737 (LD_{50})	Kimura et al. 1971
4	Mouse	(G)	1 d				4,044 (LD_{50})	Tanii et al. 1986
Systemic								
5	Rat	(G)	1 d	Hepatic Renal	1,080	1,080 (tubular necrosis)		Brown and Hewitt 1984
6	Rat	(GO)	1 d 1x/d	Hepatic	1,080			Hewitt et al. 1990
7	Rat	(GW)	3 d 1x/d	Hepatic	1,130			Raunio et al. 1990
8	Rat	(GW)	1-7 d 1x/d	Hepatic	1,500			Robertson et al. 1989
9	Rat	(G)	1 d 1x/d	Hepatic	1,500			Traiger et al. 1989
10	Rat	(GO)	1 d 1x/d	Hepatic	1,080			Brady et al. 1989
Neurological								
11	Rat	(G)	1 d				3,670 (CNS depression)	Stillmeadow Inc. 1978

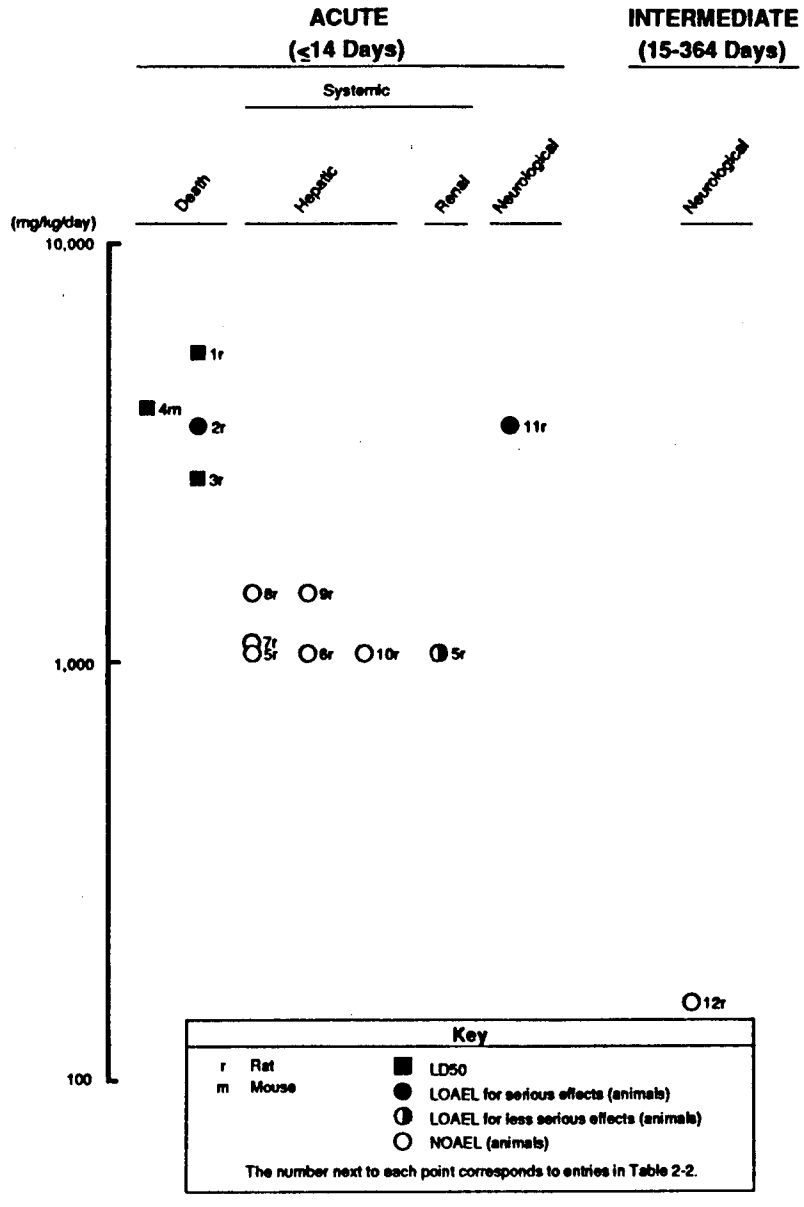
TABLE 2-2 (Continued)

Key to figure[a]	Species	Route	Exposure frequency/ duration	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
INTERMEDIATE EXPOSURE								
Neurological								
12	Rat	(G)	13 wk 5 d/wk		173			Ralston et al. 1985

[a]The number corresponds to entries in Figure 2-2.

CNS = central nervous system; d = day; (G) = gavage (undiluted); LD_{50} = lethal dose 50% kill; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; wk = week(s)

FIGURE 2-2. Levels of Significant Exposure to 2-Butanone - Oral



uneventful recovery over the next few days, and was discharged after 1 week(Kopelman and Kalfayan 1983).

All albino rats receiving 3,670 mg/kg or more had labored breathing, and most of them died within 1 hour (Stillmeadow Inc. 1978). It is'not clear whether the labored breathing represented a respiratory or a neurological response to a high dose. No other studies were located regarding respiratory effects after oral exposure to 2-butanone.

Cardiovascular effects. Cardiovascular effects observed in a 47-year-old woman after accidental ingestion of 2-butanone were decreased blood pressure and increased pulse rate (Kopelman and Kalfayan 1983). No other reports were located regarding cardiovascular effects in humans following oral exposure to 2-butanone.

No studies were located regarding cardiovascular effects in animals following oral exposure to 2-butanone.

Hepatic Effects. No studies were located regarding hepatic effects in humans following oral exposure to 2-butanone.

2-Butanone had no effect on liver weight, SGPT, or serum ornithine carbamyl transferase activities measured 42 hours after oral exposure of rats to 1,080 mg/kg (Hewitt et al. 1983). Similarly, Brown and Hewitt (1984)observed normal SGPT activity in rats exposed orally to 1,080 mg 2-butanone/kg. Although histological examination was not performed, 2-butanone appears to have a low order of hepatic toxicity in inhalation studies. Several studies have shown that 2-butanone has the ability to induce microsomal liver enzymes. Acute oral treatment of rats with 2-butanone at doses of 1,080 to 1,500 mg/kg/day for l-7 days resulted in increased levels of cytochrome P-450, increased activities of cytochrome P-450-dependent monooxygenases (Brady et al. 1989; Raunio et al. 1990; Robertson et al. 1989; Traiger et al. 1989) and proliferation of the smooth endoplasmic reticulum(Traiger et al. 1989). In the absence of clinical or histological evidence of liver damage, induction of microsomal enzymes probably represents a normal physiological response to xenobiotics rather than an adverse effect. Furthermore, oral treatment of rats with 1,080 mg/kg 2-butanone had no effect on the fragility of hepatic lysosomes or on the calcium uptake by mitochondria or microsomes (Hewitt et al. 1990). Therefore, the doses of 1,080-1,500 mg/kg can be considered acute oral NOAEL values for hepatic effects.

Renal Effects. No studies were located regarding renal effects in humans following oral exposure to 2-butanone.

Oral exposure of rats to 1,080 mg 2-butanone/kg caused mild renal tubular necrosis but had no effect on renal organic ion transport(PAH, TEA) or plasmacreatinine (Brown and Hewitt 1984). No other studies were located regardingrenal effects in animals after oral exposure to 2-butanone.

2.2.2.3 Immunological Effects

No studies were located regarding immunological effects in humans or animals after oral exposure to 2-butanone.

2.2.2.4 Neurological Effects

No studies were located regarding neurological effects in humans after oral exposure to 2-butanone.

In animals, clinical signs of central nervous system toxicity including lethargy, labored breathing, ptosis, lacrimation, exophthalmos, ataxia, salivation, and piloerection were observed in rats treated by gavage with 2-butanone at doses greater than or equal to 3,670 mg/kg (Stillmeadow Inc.1978). Most of these rats died. No effect was observed on neurobehavioral tests including hindlimb grasp, hindlimb place, balance beam, and roto-rod in rats treated by gavage with 2-butanone at a time-weighted average dose of 173 mg/kg/day for 90 days (Ralston et al. 1985). No other studies were located regarding neurological effects in animals after oral exposure to 2-butanone. The NOAEL value and LOAEL value for neurological effects are recorded in Table 2-2 and plotted in Figure 2-2.

No studies were located regarding the following health effects in humans or animals after oral exposure to 2-butanone:

2.2.2.5 Developmental Effects
2.2.2.6 Reproductive Effects
2.2.2.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.4.

2.2.2.8 Cancer

No studies were located regarding cancer in humans or animals after oral exposure to 2-butanone.

2.2.3 Dermal Exposure

2.2.3.1 Death

No studies were located regarding death in humans after dermal exposure to 2-butanone. One study reported the dermal LD_{50} for 2-butanone in rabbits to be greater than 10 mL/kg (Smyth et al. 1962). No other studies were located regarding death in animals after dermal exposure to 2-butanone.

2.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, or renal effects in humans or animals after dermal exposure to 2-butanone.

The only systemic effects of dermal exposure studied were dermal and ocular. The highest NOAEL value and all reliable LOAEL values for dermal and ocular effects in each species and duration category are recorded in Table 2-3.

Dermal/Ocular Effects. Application of 0.1 mL undiluted 2-butanone once daily for 18 days to the volar forearm of volunteers did not result in erythema, increase in skin-fold thickness, or edema over the 18-day exposure period Wahlberg (1984). Further details regarding the number of volunteers were not reported.

In rabbits and guinea pigs, application of undiluted 2-butanone caused minimal skin irritation, erythema, and/or increase in skin-fold thickness (Anderson et al. 1986; Hazleton Laboratories 1963a; Wahlberg 1984). Slight desquamation occurred in guinea pigs after 31 weeks of dermal exposure to increasing amounts of 2-butanone (Eastman Kodak 1978). Abraded skin areas were slightly more sensitive to the application of 2-butanone (Hazleton Laboratories 1963a).

2-Butanone instilled into the conjunctival sac of rabbits caused irritation, cornea1 opacity, and conjunctivitis (Davis and Baker 1975; Haskell Laboratories 1971; Hazleton Laboratories 1963b; Kennah et al. 1989). These effects were generally reversible in 7-14 days. Hazleton Laboratories (1963b) reported that one of six rabbits had persistent cornea1 damage after 7 and 14 days. On the basis of Draize scores in these studies, 2-butanone was classified as moderately irritating.

2.2.3.3 Immunological Effects

One clinical report of 2-butanone-evoked contact urticaria was located. A 48-year-old man employed as a painter complained of severe irritation when he handled 2-butanone (Varigos and Nurse 1986). A small amount of 2-butanone applied to his forearm produced a bright red area at the site of application. The area became itchy, but no induration or edema was noted. After 15 minutes, the reaction subsided. Two days later, the test was repeated with the same result. Five volunteers were later tested for sensitivity to 2-butanone by the same method, but no response was observed.

No studies were located regarding immunological effects in animals after dermal exposure to 2-butanone.

TABLE 2-3. Levels of Significant Exposure to 2-Butanone - Dermal

Species	Exposure frequency/ duration	System	NOAEL	LOAEL (effect) Less serious	LOAEL (effect) Serious	Reference
ACUTE EXPOSURE						
Systemic						
Rabbit	24 hr	Derm/oc		0.5 mL (erythema)		Hazleton Laboratories 1963a
Rabbit	1 d	Derm/oc			0.1 mL (corneal damage)	Hazleton Laboratories 1963b
Rabbit	1 d	Derm/oc	0.03 mL	0.1 mL (irritation, corneal thickening)		Kennah et al. 1989
Gn pig	3 d 3/d	Derm/oc		10 $\mu L/cm^2$ (erythema)		Anderson et al. 1986
Gn pig	10 d 1/d	Derm/oc		0.1 mL (skin-fold thickening)		Wahlberg 1984
INTERMEDIATE EXPOSURE						
Systemic						
Human	18 d 1/d	Derm/oc	0.1 mL			Wahlberg 1984

d = day; Derm/oc = dermal/ocular; Gn pig = guinea pig; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level

2.2.3.4 Neurological Effects

No studies were located regarding neurological effects in humans after dermal exposure to 2-butanone.

In an intermediate study of dermal exposure, l-2 mL of undiluted 2-butanone was applied in increasing amounts to shaved areas on the backs of guinea pigs 5 days/week for 31 weeks or less (Eastman Kodak 1978). No clinical signs of neurotoxicity were observed. No evidence of neurotoxicity was noted on examination of Epon sections of the medulla oblongata and tibia1 nerve by light microscopy (Eastman Kodak 1978). The details of 2-butanone application, however, were not clear in this report.

No studies were located regarding the following health effects in humans or animals after dermal exposure to 2-butanone:

2.2.3.5 Developmental Effects

2.2.3.6 Reproductive Effects

2.2.3.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2-4.

2.2.3.8 Cancer

No studies were located regarding cancer in humans or animals after dermal exposure to 2-butanone.

2.3 TOXICOKINETICS

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

2-Butanone is well absorbed during inhalation exposure. Pulmonary uptake in humans ranged from 41% to 56% of the inspired quantity (Liira et al. 1988a, 1988b, 1990). Exercise increased the pulmonary uptake due to the greater ventilatory rate (Liira et al. 1988b). The high blood/air solubility ratio of 2-butanone also favors absorption (Saida et al. 1976; Perbellini et al. 1984). Several investigators have reported that exposure concentrations of Z-butanoneare significantly correlated with blood concentrations in humans (Brown et al. 1986; Brugnone et al. 1983; Ghittori et al. 1987; Liira et al. 1988a, 1988b;Lowry 1987; Miyasaka et al. 1982; Perbellini et al. 1984; Tolos et al. 1987). Exposure of humans to 200 ppm 2-butanone for 4 hours resulted in blood concentrations of 3.5-7.2 μg/mL (Liira et al. 1988a, 1988b; Lowry 1987). Occupational concentrations are significantly correlated with blood and urine concentrations of unmetabolized 2-butanone (Brugnone et al. 1983; Ghittori et al. 1987; Miyasaka et al. 1982). Blood levels of 2-butanone are also

significantly correlated with breath levels (Brown et al. 1986). These data indicate that 2-butanone is absorbed upon inhalation.

Information on the absorption of 2-butanone by animals after inhalation exposure is limited. Rats that were exposed to 600 ppm 2-butanone for 6 hours on 1 day or for 6-10 hours/day for 8 days had blood concentrations of 1,041 μmol/L after a single exposure and 1,138 μmol/L after repeated exposure(Liira et al. 1991). The similarity in blood concentrations after single and repeated intermittent exposure indicates that 2-butanone does not accumulate.

2.3.1.2 Oral Exposure

A woman who had metabolic acidosis after having accidentally ingested 2-butanone stored in a rum bottle had a blood concentration of 95 mg/lOO mL(13.2 mM) (Kopelman and Kalfayan 1983). A man who intentionally ingested 100 mL of liquid cement containing a mixture of acetone (18%), 2 butanone (28% or about 37 mg/kg), and cyclohexanone (39%) had a plasma level of 2-butanone of about 110 μg/mL at 5 hours after ingestion (Sakata et al. 1989). These reports provide qualitative evidence that 2-butanone is absorbed following oral exposure in humans, but do *not* provide information regarding the extent of absorption. In the first case, the quantity ingested was unknown, while in the second case, the man was treated by gastric lavage at 2 hours after ingestion.

Oral administration (gavage) of 1,690 mg 2-butanone/kg in rats resulted in a plasma concentration of 94 mg/lOO mL at 4 hours (Dietz and Traiger 1979). Within 18 hours, the plasma concentration decreased to 6.2 mg/lOO mL (Dietzand Traiger 1979). A second, similar experiment in rats showed that, after oral administration of 1,690 mg 2-butanone/kg, the plasma concentration was 95 mg/lOO mL; the concentration decreased to 7 mg/lOO mL by 18 hours (Dietz et al. 1981). These data indicate that 2-butanone is rapidly absorbed and eliminated after oral administration.

2.3.1.3 Dermal Exposure

No studies were located regarding the rate or extent of absorption of 2-butanone in humans or animals following dermal exposure.

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

No studies were located regarding the distribution of 2-butanone following inhalation exposure in humans.

In vitro determinations of the 2-butanone tissue/air solubility ratio for human kidney, liver, muscle, lung; heart, fat, and brain show that the solubility is similar in all tissues, and that the ratio is nearly equal to

200 (Perbellini et al. 1984). Blood/tissue solubility ratios are all near unity; therefore, 2-butanone is not expected to concentrate in any one tissue(Perbellini et al. 1984).

Information regarding distribution of 2-butanone in animals after inhalation exposure is limited. Rats that were exposed to 600 ppm 2-butanone for 6 hours on 1 day or for 6-10 hours/day for 8 days had blood concentrations of 1,041 μmol/L after a single exposure and 1,138 μmol/L after repeated exposure (Liira et al. 1991). The concentration of 2-butanone in perirenal fat was 0.71 μmol/g after a single exposure and 0.70 μmol/g after repeated exposure. The similarity in blood and perirenal concentrations after single and repeated intermittent exposure indicates that 2-butanone does not accumulate.

2.3.2.2 Oral Exposure

No studies were located regarding the distribution of 2-butanone following oral exposure in humans or animals.

2.3.2.3 Dermal Exposure

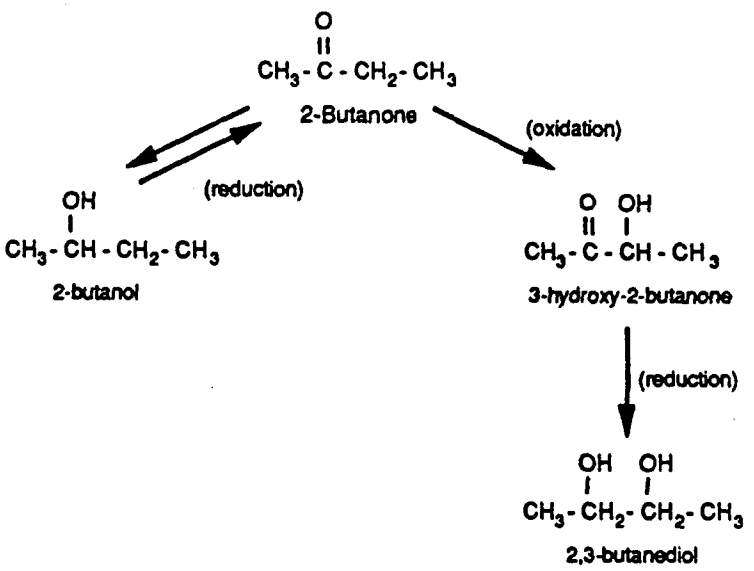
No studies were located regarding the distribution of 2-butanone following dermal exposure in humans or animals.

2.3.3 Metabolism

Few studies exist regarding the metabolism of 2-butanone in humans. Two metabolites of 2-butanone have been identified in human urine after inhalation exposure. They are 3-hydroxy-2-butanone (Brugnone **et** al. 1983; Perbellini et al. 1984) and 2,3-butanediol (Liira et al. 1988a, 1988b, 1990). The urinary concentrations of these metabolites, however, represent only about 0.1%-2% of the absorbed 2-butanone. P-Butanol was found in the blood of male volunteers exposed to 200 ppm 2-butanone for 4 hours (Liira et al. 1990). In addition to 3-hydroxy-2-butanone and 2,3-butanediol, a third metabolite, 2-butanol, has been found in the blood in guinea pigs (DiVincenzo et al. 1976)and rats (Dietz et al. 1981). About 30% of the 2-butanone administered orally in rats was converted to 2,3-butanediol; 4% was converted to 2-butanol, and 4% was converted to 3-hydroxy-2-butanone (Dietz et al. 1981).

In guinea pigs, 2-butanone was metabolized by both oxidative and reductive pathways (Figure 2-3). Oxidation produces 3-hydroxy-2-butanone, which is then reduced to 2,3-butanediol (DiVincenzo et al. 1976). Reduction of 2-butanone produces 2-butanol. The metabolites of 2-butanone in guinea pigs were excreted in the urine as 0-glucuronides or O-sulfates.

FIGURE 2-3. Proposed Metabolic Pathways for 2-Butanone*



*Adapted from DiVincenzo et al. 1976

2.3.4 Excretion

2.3.4.1 Inhalation Exposure

Urinary excretion of unchanged 2-butanone and its metabolites, 3-hydroxy-2-butanone and 2,3-butanediol, accounts for only 5% or less of the 2-butanone absorbed by inhalation in humans (Liira et al. 1988a, 1990;Perbellini et al. 1984). Unchanged 2-butanone is excreted primarily through the lungs; the quantity eliminated by this route is an estimated 20X-40% (Browning 1965; Riihimaki 1986); however, only about 3% of absorbed 2-butanone was excreted unchanged in the expired air of humans exposed to 200 ppm for 4 hours (Liira et al. 1988a, 1990). 2-Butanone is rapidly cleared from the blood with a reported plasma half-life in humans of 49-96 minutes (Brown et al. 1986; Liira et al. 1988a; Lowry 1987) and an apparent clearance rate of 0.60 L/minute (Liira et al. 1990). Therefore, 2-butanone would not be expected to accumulate with chronic exposure (Lowry 1987).

No studies were located regarding excretion of 2-butanone in animals after inhalation exposure.

2.3.4.2 Oral Exposure

Information regarding the excretion of 2-butanone after oral exposure in humans is limited. A man who intentionally ingested 100 mL of liquid cement containing a mixture of acetone (18X), 2-butanone (28% or about 37 mg/kg), and cyclohexanone (39%) had a plasma level of 2-butanone of about 110 μg/mL at 5 hours after exposure (Sakata et al. 1989). The plasma level declined to about 95 μg/mL at 12 hours and to <20 μg/mL at 18 hours, where it remained until about 25 hours and slowly declined to <5 μg/mL at 48 hours. Urine levels of 2-butanone decreased gradually from 123 μg/mL at 5 hours to 61 μg/mL at 19 hours. Disappearance from the urine then became more rapid with about 10 μg/mL excreted at 48 hours. While this study provided information on the elimination of 2-butanone from plasma and urine of a human orally exposed, coexposure to the other components of the cement could have influenced the elimination.

No studies were located regarding the rate or extent of excretion of 2-butanone in animals following oral exposure.

2.3.4.3 Dermal Exposure

No studies were located regarding the rate or extent of excretion of 2-butanone in humans or animals following dermal exposure.

2.4 RELEVANCE TO PUBLIC HEALTH

The only known effects of 2-butanone in humans are related to its irritating properties on the respiratory and dermal/ocular systems. Effects

observed in animals include death, irritation of respiratory tissue, eyes, and skin, liver congestion, kidney congestion, cornea1 opacity, narcosis and incoordination, and fetotoxicity.

No acute-, intermediate-, or chronic-duration inhalation MRLs were derived for 2-butanone. In the case of acute-duration inhalation exposure, target organs have not been sufficiently identified. Intermediate-duration inhalation studies likewise failed to identify target organs, and nose and throat irritation occurred in humans exposed for 5 minutes to exposure levels that were much lower than NOAEL values in animals in intermediate-duration studies. No studies were located regarding toxic effects in humans or animals after chronic inhalation exposure, precluding the derivation of a chronic inhalation MRL. No acute, intermediate-, or chronic-duration oral KRLs were derived for 2-butanone. In the case of acute-duration oral exposure, target organs have not been sufficiently identified. The paucity of information on toxic effects after intermediate- and chronic-duration oral exposure likewise precludes the derivation of MRLs for these durations. Acute-duration, intermediate-duration, and chronic-duration dermal MRLs were not derived for 2-butanone due to the lack of appropriate methodology for the development of dermal MRLs.

Death. No studies were located regarding death of humans after inhalation, oral, or dermal exposure to 2-butanone. Death of rats and mice occurred within a few hours during acute inhalation exposure to very high concentrations (greater than or equal to 90,000 ppm) (Klimisch 1988; LaBelle and Brieger 1955). The inhalation 4-hour LC_{50} in rats was 11,700 ppm (LaBelle and Brieger 1955). In the intermediate-duration studies, no rats died after exposure to 5,000 ppm or less for 6 hours/day, 5 days/week for 90 days(Cavender et al. 1983), but all rats exposed to 6,000 ppm, 8 hours/day, 7 days/week died from bronchopneumonia (Altenkirch et al. 1978a, 1978b). The bronchopneumonia may have been caused by the 2-butanone exposure because the results were reproducible and did not occur in rats exposed to n-hexane or the combination of 2-butanone and n-hexane (Altenkirch et al. 1978a, 1978b). The concentration of 2-butanone that would cause death in humans after inhalation is not known. It does not seem likely that humans would be exposed to the high concentrations that are fatal to animals except in an occupational accident. 2-Butanone has a half-life in air of only 14 hours; therefore, in the vicinity of toxic waste sites, ambient concentrations would be expected to be low.

Information regarding death from oral and dermal exposure is limited to LD_{50} determinations. Oral LD_{50} values have been reported to be approximately 2,740 mg/kg *in* immature, young adult, and older Sprague-Dawley rats (Kimura et al. 1971), 5,542 mg/kg in Wistar rats (Smyth et al. 1962), and 4,044 mg/kg in mice (Tanii et al. 1986). The dermal LD_{50} in rabbits is greater than 10 mL/kg (Smyth et al. 1962). Oral exposure of humans to 2-butanone might occur through drinking water if this chemical seeped from a waste site, for example, into the groundwater. 2-Butanone is highiy water soluble and is

expected to have a high soil mobility. Exposure through drinking water is,therefore, possible, but fatal concentrations are unlikely Dermalexposure of humans is unlikely to result in death.

Systemic Effects. There are few known systemic effects of 2-butanone exposure in humans. Three men exposed to 2-butanone vapors while removing paint from an airplane hangar developed mild respiratory symptoms; however, the nature and extent of these symptoms were not described (Berg 1971). One of the men suffered a loss of vision secondary to retrobulbar neuritis, but this was reversed within 36 hours. Furthermore, exposure to methanol could not be ruled out. Nelson et al. (1943) reported that 100 ppm 2-butanone caused slight nose and throat irritation, and that some subjects complained of mild eye irritation at 200 ppm. Subjects could not tolerate 350 ppm 2-butanone. For this study, it was estimated that 200 ppm would be the maximum concentration of 2-butanone tolerable for an 8-hour exposure period. No adverse effects were reported in several studies exposing volunteers to 200 ppm 2-butanone for 4 hours (Dick et al. 1984, 1988, 1989; Liira et al. 1988a, 1988b). In contrast, sporting goods manufacturing plant workers exposed to 250 ppm or less complained of skin, eye, nose, and throat irritation, and central nervous system symptoms (headache, dizziness, fatigue)(Lee and Murphy 1982). One worker in this group complained of "acting differently," but the change in behavior was not described. These exposures were in enclosed areas with poor ventilation. Exposure at the sporting goods factory was not to 2-butanone exclusively; other solvent vapors were also present. Therefore, the central nervous system symptoms described may not have been due solely to 2-butanone.

Respiratory Effects. The respiratory effects observed in humans are discussed above. Upper respiratory tract irritation was reported in rats exposed for a few hours to 10,000 ppm 2-butanone (Altenkirch et al. 1978a). After the concentration was lowered to 6,000 ppm, all the rats died suddenly at 7 weeks. Bronchopneumonia was confirmed pathologically as the cause of death. In contrast, no respiratory tract irritation or infection was observed in rats,exposed to 5,000 ppm 2-butanone for 90 days (Cavender et al. 1983). Patty et al. (1935) reported that acute inhalation exposure of guinea pigs to 10,000 ppm or more of 2-butanone produced gasping respiration, emphysematous lungs, ocular irritation, and lacrimation. Exposure to 100,000 ppm for 30 minutes or more caused cornea1 opacity, a condition which gradually improved in guinea pigs that lived 8 days after exposure. Since 2-butanone exposure is not tolerable to humans at concentrations of 350 ppm (Nelson et al. 1943), it is highly unlikely that inhalation exposure could result in respiratory, dermal, or ocular effects more serious than minor irritation. Dermal exposure of humans, rabbits, and guinea pigs has produced irritation to the skin (Anderson et al. 1986; Hazleton Laboratories 1963a; Wahlberg 1984). Intraocular exposure of rabbits has resulted in cornea1 damage (Hazleton Laboratories 1963b; Kennah et al. 1989). Dermal and eye contact with liquid 2-butanone is possible in occupational settings and at hazardous waste sites.

Hepatic Effects. No studies were located regarding hepatic effects in humans after inhalation, oral, or dermal exposure to 2-butanone. Animal data indicate that hepatic effects after high-level exposure to 2-butanone would be minimal in humans. Liver congestion was found in guinea pigs exposed acutely by inhalation to 10,000 ppm or more (Patty et al. 1935). Serum concentrations of hepatic enzymes were not changed in rats after 2-butanone exposures of 300-5,000 ppm for l-12 weeks (Cavender et al. 1983; Li et al. 1986; Schwetz et al. 1974). No lesions that could be linked to 2-butanone exposure were found following histological examination, although a slight increase in absolute and relative liver weight was noted (Cavender et al. 1983). After exposure of rats to 800 ppm 2-butanone for 5 weeks, no changes were observed in the content of hepatic cytochrome P-450 or in the cytochrome P-450 isozyme profile (Toftgard et al. 1981). 2-Butanone, however, altered the metabolism of androstenedione by increasing the formation of two metabolites and decreasing the formation of two other metabolites. Furthermore, liver weight was increased in the 2-butanone-exposed rats (Toftgard et al. 1981). While no induction of microsomal enzymes was found in rats exposed to 2-butanone by inhalation, several studies have shown that E-butanone has the ability to induce microsomal liver enzymes in rats after acute oral exposure (Brady et al. 1989; Raunio et al. 1990; Robertson et al. 1989; Traiger et al. 1989). These studies also suggested that 2-butanone potentiates the toxicity of other chemicals, such as, carbon tetrachloride, n-hexane, m-xylene, and chloroform, by increasing their metabolism to toxic metabolites. While enzyme induction by itself represents a normal physiological response to a xenobiotic rather than an adverse effect, the enzyme induction by 2-butanone can be viewed as an adverse effect if coexposure to other chemicals for which 2-butanone potentiates toxicity by this mechanism occurs. Therefore, humans working or living near hazardous waste sites where these chemicals are present along with 2-butanone may be at greater risk of adverse hepatic effects.

Renal Effects. Renal effects of 2-butanone exposure in humans would probably be minimal based on animal data. Kidney congestion was found in guinea pigs exposed acutely by inhalation to 10,000 ppm or more (Patty et al. 1935). Cavender et al. (1983) assessed kidney function with measurements of blood urea nitrogen, urine volume, urine specific gravity, and pH after a go-day exposure to 5,000 ppm 2-butanone. All values were within normal ranges, and no histopathological lesions attributable to 2-butanone exposure were found. Oral exposure of rats to 1,080 mg 2-butanone/kg caused mild renal tubule necrosis but had no effect on renal organic ion transport or plasma creatinine; therefore, in spite of mild necrosis, normal kidney functions were not impaired. Exposure of humans to 2-butanone at hazardous waste sites is, therefore, not likely to result in severe kidney effects.

Neurological Effects. The main neurological complaints of humans exposed occupationally to 2-butanone are headaches, dizziness, nausea, and fatigue (Lee and Frederick 1981; Lee and Parkinson 1982). However, these symptoms were not reported in several inhalation studies in which humans were exposed to 200 ppm of 2-butanone for 4 hours (Dick et al. 1984, 1988, 1989; Liira

et al. 1988a, 1988b). In the occupational exposures cited, 2-butanone was combined with several other solvents; therefore, a neurological effect exclusive to 2-butanone cannot be inferred. Dick et al. (1984, 1988, 1989) found that exposure to 2-butanone had no effect on any neurobehavioral measurement. Nevertheless, early signs of narcosis and incoordination based on a battery of neurobehavioral tests were described in juvenile baboons after continuous exposure to 100 ppm 2-butanone for 7 days (Geller et al. 1979). It is also possible that the baboons were distracted during testing by the irritant effects of 2-butanone on the respiratory tract and were not, as the investigators concluded, in a state of narcosis.

Narcosis and incoordination were also observed in guinea pigs exposed to 10,000 ppm or more 2-butanone in air for a few hours (Patty et al. 1935). 2-Butanone was not neurotoxic at lower concentrations in longer duration studies in animals. Rats continuously exposed to 1,125 ppm for 5 months showed no signs of peripheral neuropathy on histological examination (Saida et al. 1976). Altenkirch et al. (1978a) observed no clinical signs of neuropathy in rats exposed for 7 weeks to 6,000 ppm. No neurological effects were observed in rats exposed by inhalation to\ 5,000 ppm for 90.days (Cavender et al. 1983). Alterations in nerve conduction velocity were seen in rats exposed by inhalation for 4 weeks to 200 ppm 2-butanone (Takeuchi et al. 1983). The toxicological significance of this observation is questionable because normal nerve conduction velocities were observed at all time points beyond 4 weeks. No neurological effects were observed in rats after oral exposure to 1,725 mg/kg for 90 days (Ralston et al. 1985). Therefore, exposure of humans to 2-butanone alone in the workplace or at hazardous waste sites is not likely to result in serious neurological effects.

Although exposure to 2-butanone appears relatively innocuous, this ketone is very hazardous in combination with other solvents. 2-Butanone markedly potentiates the neurotoxicity of ethanol, n-hexane, methyl-n-butyl ketone, and ethyl-n-butyl ketone (Altenkirch et al. 1977; Cunningham et al. 1989; King et al. 1985; Ralston et al. 1985; Robertson et al. 1989; Vallat et al. 1981). Glue formulations containing both 2-butanone and n-hexane caused "glue sniffers' neuropathy" (Altenkirch et al. 1977; King et al. 1985; Vallat et al. 1981). This neuropathy is characterized by motor nerve dysfunction, paresis, paralysis, muscular atrophy, and neural tissue morphology changes including paranodal axon swelling, neurofilamentous hyperplasia, and demyelination (see Section 2.6). Therefore, humans working or living near hazardous waste sites where methyl-n-butyl ketone, ethyl-n-butyl ketone, or n-hexane is present or who frequently use alcohol may be at greater risk for neurological effects if 2-butanone is also present.

Developmental Effects. No studies were located regarding developmental effects in humans following inhalation, oral, or dermal exposure to 2-butanone. Inhalation exposure of rats and mice to 3,000 ppm during gestation resulted in fetotoxic effects, such as reduced fetal weight, skeletal variations, and delayed ossification (Deacon et al. 1981; Mast et al.

1989; Schwetz et al. 1974). It is not known whether exposure of humans to 2-butanone by any route would result in fetotoxic effects, but the presence of these effects in two animal species strongly suggests that such effects might occur in humans.

Reproductive Effects. No studies were located regarding reproductive effects in humans following inhalation, oral, or dermal exposure to 2-butanone. The only study regarding reproductive effects in animals was a go-day inhalation study in rats exposed to 5,000 ppm or less of 2-butanone (Cavender and Casey 1981; Cavender et al. 1983). Histological examination of male and female reproductive organs revealed no effects, but reproductive function was not tested.

Genotoxic Effects. *In vivo* and in vitro studies regarding the genotoxicity of 2-butanone are summarized in Table 2-4. No induction of micronuclei was found in the erythrocytes of mice (O'Donoghue et al. 1988) or hamsters (Basler 1986) after intraperitoneal injection with 2-butanone. &I vitro studies sponsored by the Chemical Manufacturers' Association showed that 2-butanone was not mutagenic in the Salmonella/mammalian-microsome preincubation mutagenicity assay (Ames test) or the L5178Y TK +/- mouse lymphoma mutagenesis assay with or without activation (O'Donoghue et al. 1988). 2-Butanone did not induce unscheduled DNA synthesis in rat primary hepatocytes, and it did not transform BALB/3T3 cells. 2-Butanone did not increase the reverse mutation frequency in Escherichia coli or Salmonella tvohimurium, with or without activation, and did not increase the frequency of chromatid gaps, chromatid breaks, or total chromatid aberrations in rat liver cells (Thorpe 1982). 2-Butanone did not cause gene mutations in Saccharomvces cerevisiae (Thorpe 1982), but cause mitotic chromosome loss (Whittaker et al. 1990; Zimmermann et al. 1989) and aneuploidy in S. cerevisiae (Mayer and Goin 1987) at high concentrations. The positive induction of chromosome loss in the yeast cells was enhanced by coexposure to 2-butanone, ethyl acetate, and propionitrile (Zimmermann et al. 1989). The positive induction of aneuploidy was enhanced by coexposure to 2-butanone and nocodazole (Mayer and Goin 1987). It appears, therefore, that 2-butanone alone is not genotoxic to humans.

Cancer. Two retrospective epidemiological studies of industrial workers chronically exposed to 2-butanone in dewaxing plants reported that deaths due to cancer were less than expected (Alderson and Rattan 1980; Wen et al. 1985).

No other studies were located regarding cancer in humans or animals following inhalation exposure to 2-butanone.

TABLE 2-4. Genotoxicity of 2-Butanone *In Vivo* and *In Vitro*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
In vivo:				
Mouse	Micronucleated erythrocytes	Not applicable	-	O'Donoghue et al. 1988
Hamster	Micronucleated erythrocytes	Not applicable	-	Basler 1986
In vitro:				
Prokaryotic organisms:				
Salmonella typhimurium	Gene mutation	-	-	Thorpe 1982
S. typhimurium	Gene mutation	-	-	O'Donoghue et al. 1988
Escherichia coli	Gene mutation	-	-	Thorpe 1982
Eukaryotic organisms:				
Fungi:				
Saccharomyces cerevisae	Gene mutation	-	-	Thorpe 1982
S. cerevisiae	Mitotic chromosome loss	No data	+	Whittaker et al. 1990; Zimmerman et al. 1989
S. cerevisiae	Aneuploidy	No data	+	Mayer and Goin 1987
Mammalian cells:				
Rat liver cells (RL_4)	Chromosomal aberrations	No data	-	Thorpe 1982
Rat hepatocytes	Unscheduled DNA synthesis	No data	-	O'Donoghue et al. 1988
BALB/3T3	Morphological transformation	No data	-	O'Donoghue et al. 1988
Mouse lymphoma	Gene mutation	-	-	O'Donoghue et al. 1988

- = negative result; + = positive results

2.5 BIOMARRERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism(NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time biologic samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to 2-butanone are discussed in Section 2.5.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are often not substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by 2-butanone are discussed in Section 2.5.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, biologically effective dose, or target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.7, "POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE."

2.5.1 Biomarkers Used to Identify and/or Quantify Exposure to 2-Butanone.

Inhalation exposure to 2-butanone correlates well with blood, breath, and urinary concentrations of unchanged 2-butanone (Brown et al. 1986; Brugnone et al. 1983; Ghittori et al. 1987; Miyasaka et al. 1982). Personal dosimetry was used to measure exposure to 2-butanone among 62 printing plant workers(Miyasaka et al. 1982) and 659 workers in plastic boat, chemical, plastic button, paint, and shoe factories (Ghittori et al. 1987). The correlation between exposure levels and urinary concentration of unchanged 2-butanone was strong in each study (r=O.774 and r=0.91, respectively). Miyasaka et al. 1982) concluded, however, that estimating exposure from urinary levels was reliable on a group basis but not an individual basis. Blood and breath levels of 2-butanone were significantly correlated (r=0.78, p<O.OOl) in volunteers exposed to 200 ppm 2-butanone for 4 hours (Brown et al. 1986). A significant correlation between workroom and urinary 2-butanone concentrations was observed in shoe factory workers (r=0.6877, p<O.OOl) (Brugnone et al. 1983). In the same study, a more significant correlation was observed between workroom concentrations and a 2-butanone urinary metabolite, 3-hydroxy-2-butanone (r=0.8179, p<O.OOl). Another 2-butanone metabolite, 2,3-butanediol, has also been identified in the urine of humans (Liira et al. 1988a, 1988b). No studies were located regarding the correlation between exposure to 2-butanone and urinary levels of the metabolite, 2,3-butanediol. A third metabolite, 2-butanol, was identified in guinea pig-blood; however, no attempt was made to correlate 2-butanol blood levels with exposure to 2-butanone (DiVincenzo et al. 1976). Metabolism of alcohols, hydrocarbons, and other ketones may also yield 2-butanone, 3-hydroxy-2-butanone, and 2,3-butanediol (Dietz and Traiger 1979; Tsukamoto et al. 1985a); therefore, these compounds may confound assessment of exposure to 2-butanone.

Measurements of tissue, blood, and excreta levels may not be an accurate indication of past exposure to 2-butanone. Accumulation in target tissues does not occur because tissue/blood solubility ratios are all near unity; therefore, 2-butanone will not concentrate in specific tissues (Perbellini et al. 1984). The serum half-life of 2-butanone in humans is very short;estimates range from 49 to 96 minutes (Liira et al. 1988a; Lowry 1987). Furthermore, 2-butanone was not detectable in blood or breath measurements reported the morning after a 4-hour exposure to 200 ppm (Brown et al. 1987).

No quantifiable effects that could be used as biomarkers of exposure to 2-butanone were identified.

2.5.2 Biomarkers Used to Characterize Effects Caused by 2-Butanone

2-Butanone induces hepatic microsomal enzymes in rats after oral exposure(Brady et al. 1989; Raunio et al. 1990, Robertson et al. 1989; Traiger et al. 1989), but this enzyme induction has not been associated with more severe liver effects. No other subtle biochemical effects of 2 butanone have been

identified that would be useful as biomarkers to characterize effects of 2-butanone.

2.6 INTERACTIONS WITH OTHER CHEMICALS

The neurological and hepatic effects of 2-butanone alone are minimal. For certain applications, 2-butanone is combined with other chemicals that have serious neurotoxic or hepatotoxic effects. Clinical reports and animal studies have clearly shown that 2-butanone potentiates both the neurotoxicity of ethanol, n-hexane, and methyl-n-butyl ketone and the hepatotoxicity of carbon tetrachloride and chloroform.

Altenkirch et al. (1977) investigated a large outbreak of toxic polyneuropathies in a group of West Berlin "glue sniffers." The development of neuropathies (muscular atrophy, paresthesia, paresis, quadriplegia) coincided with a change in the composition of a glue that was popular for sniffing. Until the fall of 1975, the major constituents of the glue were n-hexane, toluene, ethyl acetate, and benzene. At this time, 2-butanone was added to the mixture and the sudden appearance of the toxic neuropathies began.

A 39-year-old woman who had worked for several years gluing shoes developed polyneuropathy after a few weeks of work in a poorly ventilated shop (Vallat et al. 1981). The glue she was using contained 20% 2-butanone and 8% n-hexane.

"Glue sniffing neuropathy" was also described in a clinical case report of three men who had similar symptoms (King et al. 1985). All had been sniffing the same brand of glue containing light, volatile hydrocarbons(Ce-C,), toluene, and 2-butanone. P-Butanone had recently been added to the glue formulation, and the authors suggested that this may have increased the neurotoxicity. A change in the formulation of a *solvent* compound also precipitated a sudden outbreak of peripheral neuropathy in a coated fabrics plant (Allen et al. 1975; Billmaier et al. 1974). Methyl-n-butyl ketone introduced into a solvent used at the plant was implicated as the causative agent; however, the solvent also contained high concentrations of 2-butanone. Combined exposure to 2-butanone and methyl-n-butyl ketone has not been studied meepidemiologically in humans; therefore, whether P-butanone potentiates methyl-n-butyl ketone neurotoxicity in humans is not known (Katz 1985).

The potentiation of the neurotoxicity of n-hexane and methyl-n-butyl ketone by 2-butanone is well-documented in animals (Altenkirch et al. 1978a, 1978b, 1982a, 1982b; Saida et al. 1976; Takeuchi et al. 1983). Altenkirch et al. (1978a) exposed rats to either 10,000 ppm n-hexane or a combination of 1,000 ppm 2-butanone and 9,000 ppm n-hexane. A summary of three experiments under these conditions showed that rats exposed to the combination of n-hexane and 2-butanone developed paresis more rapidly and in greater numbers than rats exposed to n-hexane only. In the same study, rats exposed to 6,000 ppm

2-butanone only showed no signs of neurotoxicity up to 7 weeks, when all the rats in this group died suddenly of bronchopneumonia. These results were confirmed in a second study; mixtures of 500 ppm n-hexane and 2-butanone (4:l or 3:2) or 700 ppm (5:2) caused clinical signs of neuropathy l-5 weeks earlier than 500 ppm n-hexane alone (Altenkirch et al. 1982a). Histological examination revealed morphological changes in the rats similar to those found in youths suffering from glue sniffing neuropathy, including paranodal axon swelling, accumulation of neurofilaments in the cytoplasm, and demyelination. Takeuchi et al. (1983) observed a significant decrease in motor nerve conduction velocity in rats exposed to 300 ppm n-hexane:2-butanone (1:2). In this study, motor nerve conduction velocity increased in rats exposed to 200 ppm 2-butanone alone and did not change in rats exposed to 100 ppm n-hexane alone. Male Wistar rats exposed to n-hexane or a combination of n-hexane and 2-butanone developed ultrastructural changes in the intrapulmonary nerves characteristic of hexacarbon neurotoxicity (Schmidt et al. 1984). Concomitant exposure to n-hexane and 2-butanone decreased the onset of observable neuropathological changes.

Saida et al. (1976) reported a marked potentiation of peripheral neurotoxicity when rats were exposed to methyl-n-butyl ketone:2-butanone (225:1,125 ppm). Rats exposed to methyl-n-butyl ketone only developed paralysis by 66 days. The combination caused paralysis in 25 *days,* while 2-butanone alone had no effect up to 5 months. Histological examination of neurons revealed morphological changes similar to those reported by Altenkirch et al. (1982a), which included paranodal axon swelling, accumulation of neurofilaments, and demyelination. Subcutaneous injection of methyl-n-butyl ketone with or without 2-butanone increased distal motor latency and decreased motor fiber conduction velocity in male Donryu strain rats (Misumi and Nagano 1985). The combination of the two ketones enhanced these effects.

In vitro studies support the hypothesis that 2-butanone potentiates n-hexane and methyl-n-butyl ketone neurotoxicity. Veronesi et al. (1984)observed that, in tissues cultured from fetal mouse spinal cord, dorsal root ganglia, and muscle, the combination of 2-butanone and n-hexane produced giant axonal swellings more rapidly than cultures treated with n-hexane alone. Furthermore, cultures exposed to nontoxic concentrations of n-hexane also developed giant axonal swellings when 2-butanone was administered concomitantly.

Biotransformation of both n-hexane and methyl-n-butyl ketone can produce 2,5-hexanedione (Couri et al. 1978; DiVincenzo et al. 1976; Robertson et al. 1989). This compound is the most potent neurotoxic metabolite of n-hexane and methyl-n-butyl ketone known (Katz 1985). 2-Butanone may potentiate n-hexane and methyl-n-butyl ketone neurotoxicity by enhancing their metabolic conversion to 2,5-hexanedione. Combined administration of methyl-n-butyl ketone and 2-butanone produced more 2,5-hexanedione than administration of methyl-n-butyl ketone alone (Couri et al. 1978). The concentrations of the n-hexane metabolites 2,5-hexanedione and 2,5-dimethylfuran were significantly

higher in the blood and sciatic nerve of rats pretreated by gavage with 2-butanone followed by inhalation exposure to n-hexane compared with rats exposed to n-hexane alone (Robertson et al. 1989). In addition, concomitant oral administration of 2-butanone and 2,5-hexanedione in rats reduced blood 2,5-hexanedione clearance (Ralston et al. 1985).

Ethyl-n-butyl ketone is a weak neurotoxin (O'Donoghue et al. 1984). Oral administration in rats for several weeks caused the paranodal axon swelling and neurofilamentous hyperplasia characteristic of n-hexane and methyl-n-butyl ketone neurotoxicity. Biotransformation of ethyl-n-butyl ketone produced two neurotoxic metabolites, 2,5-hexanedione and 2,5-heptanedione. 2,5-Heptanedione can be further metabolized to 2,5 hexanedione. Concomitant inhalation exposure to 700 ppm ethyl-n-butyl ketone and 700 ppm 2-butanone for 4 consecutive days caused a 2.6-fold increase in the serum concentration of 2,5-heptanedione. Oral administration of 2-butanone potentiated the development of clinical and histological signs of ethyl-n-butyl neurotoxicity.

2-Butanone has also been found to potentiate the neurotoxicity of ethanol (Cunningham et al, 1989). Mice pretreated intraperitoneally with 2-butanone followed by intraperitoneal injection of ethanol 30 minutes later showed prolonged loss of righting reflex induced by ethanol. 2-Butanone also decreased the rate of ethanol elimination in mice *in vivo* and inhibited the in vitro activity of alcohol dehydrogenase, the main mechanism for ethanol elimination. These results suggest that 2-butanone potentiated the neurotoxicity of-ethanol by inhibiting its metabolism by alcohol dehydrogenase.

2-Butanone is not a universal potentiator of hydrocarbon- and aliphatic ketone-induced neuropathies (O'Donoghue et al. 1982). Concomitant oral administration of 2-butanone and 5-nonanone did not potentiate the neurotoxicity of 5-nonanone.

2-Butanone alone is minimally neurotoxic (Altenkirch et al. 1978a; Saida et al. 1976). This compound, however, is frequently mixed with n-hexane and methyl-n-butyl ketone for various commercial and industrial applications. The previous discussion emphasizes the public health hazard of mixed solvent exposure to 2-butanone. Exposure to a mixed solvent is more likely to occur in an occupational setting or at a hazardous waste site, than exposure to 2-butanone alone.

2-Butanone alone is not highly hepatotoxic (see the discussion of Hepatic Effects in Section 2.2.1.2) but has a well-documented role in potentiating haloalkane-induced hepatotoxicity (Brown and Hewitt 1984; Dietz and Traiger 1979; Hewitt et al. 1983, 1986, 1987; Tanii et al. 1986). Intraperitoneal injection of chloroform (0.5 mL/kg) caused a nine-fold increase in rat SGPT activity (Brown and Hewitt 1984). In contrast, chloroform injection caused a 195-fold increase in rat SGPT activity if administered 18 hours after oral administration of 2-butanone. Similarly, intraperitoneal injection of

chloroform increased rat plasma ornithine carbamyl transferase activity 215-fold if given 18 hours after oral administration of 2-butanone (Hewitt et al. 1983). In addition to the doses administered, the length of time between administration of 2-butanone and the chloroform injection determined the severity of hepatotoxicity (Hewittet al. 1987). Measurements of SGPT and plasma ornithine carbamyl transferase revealed that 2-butanone is most efficacious for potentiation of chloroform-induced hepatotoxicity if administered 18 hours before chloroform.

2-Butanone also potentiates carbon tetrachloride-induced hepatotoxicity (Dietz and Traiger 1979; Traiger et al, 1989). Measurement of SGPT activity and hepatic triglyceride content showed that administration of 2-butanone 16 hours before intraperitoneal injection of carbon tetrachloride significantly enhanced liver damage. The mechanism of P-butanone potentiation of chloroform and carbon tetrachloride hepatotoxicity may be related to biotransformation of the ketone to its metabolite, 2,3-butanediol. Carbon tetrachloride increased rat SGPT 164-fold when injected 16 hours after oral administration of 2,3-butanediol. Replacement of 2,3-butanediol with 2-butanone increased the transaminase 66-fold. Hepatic triglyceride content was potentiated to a similar degree by both 2-butanone and 2,3-butanediol. However, the maximal potentiation of carbon tetrachloride-induced hepatic injury by pretreatment with 2-butanone coincided with increased microsomal enzyme activity within the same time frame following exposure to 2-butanone alone (Traiger et al. 1989). This strongly suggests that 2-butanone potentiates the hepatotoxicity of carbon tetrachloride by enhancing its metabolism to toxic intermediates. The mechanism of 2-butanone potentiation of chloroform-induced hepatotoxicity apparently does not involve biotransformation of chloroform to a reactive intermediate, an alteration of the cytochrome P-450 system, or depletion of liver glutathione (Hewitt et al. 1987). It is possible that 2,3-butanediol also contributes to the toxicity of chloroform.

Pretreatment of ddY mice with carbon tetrachloride 24 hours before oral administration of 2-butanone reduced the 2-butanone LD,, about 20% (Tanii et al. 1986). The mechanism of this effect was not investigated.

Exposure of pregnant rats continuously to n-hexane alone (l,OOO-1,500 ppm) or n-hexane and 2-butanone (1,200 ppm n-hexane, 300 ppm 2-butanone) throughout gestation and/or during the postnatal period resulted in reduced birth weight of pups, and weight gain reduction persisted during the postnatal exposure period (Stoltenburg-Didinger et al. 1990). The effect was more pronounced with the mixture of solvents. In addition, hindlimb weakness in one dam during the gestational exposure period progressing to quadriplegia in all dams during the postpartum exposure period was for the solvent mixture, while only hindlimb weakness was observed in the dams exposed observed n-hexane alone.

Coexposure of S. cerevisiae to 2-butanone, ethyl acetate, and propionitrile enhanced the induction of chromosome loss caused by 2-butanone (Zimmermann et al. 1989). Coexposure of S. cerevisiae to 2-butanone and nocodazole enhanced the induction of aneuploidy caused by 2-butanone alone (Mayer and Goin 1987).

2.7 POPULATIONS THAT ABE UNUSUALLY SUSCEPTIBIE

No studies were located that identified populations that are unusually susceptible to adverse health effects after exposure to 2-butanone. The very young and the very old are typically more susceptible to chemical, toxicity than are older children, adolescents, and healthy adults. Individuals that are alcoholics and those with existing liver disease would be expected to metabolize 2-butanone differently than the general population. Persons with existing neuropathies may also be more susceptible. Exposure to both 2-butanone and n-hexane or methyl-n-butyl ketone is possible in occupational settings and at hazardous waste sites; thus, neurological effects of n-hexane and methyl-n-butyl ketone may be greater with coexposure to 2-butanone. Likewise, occupational exposure or exposure at hazardous waste sites to a combination of 2-butanone and the haloalkanes, carbon tetrachloride, or chloroform, presents a greater risk for liver damage.

2.8 MITIGATION OF EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to 2-butanone. This section is intended to inform the public of existing clinical practice and the status of research concerning such methods. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to 2-butanone. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

2-Butanone has a low order of systemic toxicity. The main effects in humans are irritation of respiratory tissues and eyes and nonspecific neurological effects, such as headache, dizziness, nausea, and fatigue. Such effects are characteristic of solvent exposure and are mitigated primarily by removing affected individuals from exposure conditions and decontaminating exposed areas (Bronstein and Currance 1988; Stutz and Janusz 1988). For example, contaminated clothing is removed and skin washed. If the eyes were exposed, they are flushed with water. For ingestion of 2-butanone, there is controversy as to whether or not to administer emetics. The controversy centers around the risk of aspiration of vomitus into the lungs during emesis. Administration of activated charcoal has been suggested to reduce gastrointestinal absorption. Please refer to Bronstein and Currance (1988) and Stutz and Janusz (1988) for more complete information.

Although 2-butanone alone is not highly neurotoxic or hepatotoxic, it potentiates the neurotoxicity of n-hexane and methyl-n-butyl ketone, and the hepatotoxicity of chloroform and carbon tetrachloride (see Section 2.6). Exposure to 2-butanone with other solvents is more likely than exposure to 2-butanone alone in occupational and environmental settings. Since both n-hexane and methyl-n-butyl ketone can be

metabolized to 2,5-hexanedione (Couri et al. 1978; DiVincenzo et al 1976), a potent neurotoxic agent (Katz 1985), 2-butanone may potentiate the neurotoxicity of the other chemicals by enhancing the biotransformation of n-hexane and methyl-n-butyl ketone to 2,5-hexanedione by inducing microsomal enzymes. The potentiation of the hepatotoxicity of carbon tetrachloride and chloroform by 2-butanone may be related to the biotransformation of 2-butanone to 2,3-butanediol, a metabolite that potentiates the hepatotoxicity of carbon tetrachloride to a greater extent than does 2-butanone (Dietz and Traiger 1979). Alternatively, the induction of microsomal enzymes by 2-butanone may enhance the biotransformation of chloroform and carbon tetrachloride to toxic intermediates (Brady et al. 1989; Traiger et al. 1989). If carbon tetrachloride or chloroform shift metabolic pathways of 2-butanone in favor of greater formation of 2,3-butanediol, administration of an agent that blocks this shift could mitigate the potentiation. 2-Butanone is reduced to 2-butanol and oxidized to 3-hydroxy-2-butanone, which is further reduced to 2,3-butanediol(DiVincenzo et al. 1976). However, the enzyme systems involved in the biotransformation of 2-butanone have not been characterized. Similarly, if 2-butanone enhances the metabolism of chloroform and carbon tetrachloride by inducing microsomal enzymes, agents that block this induction could mitigate this potentiation.

2.9 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA as amended directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of 2-butanone is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of 2-butanone.

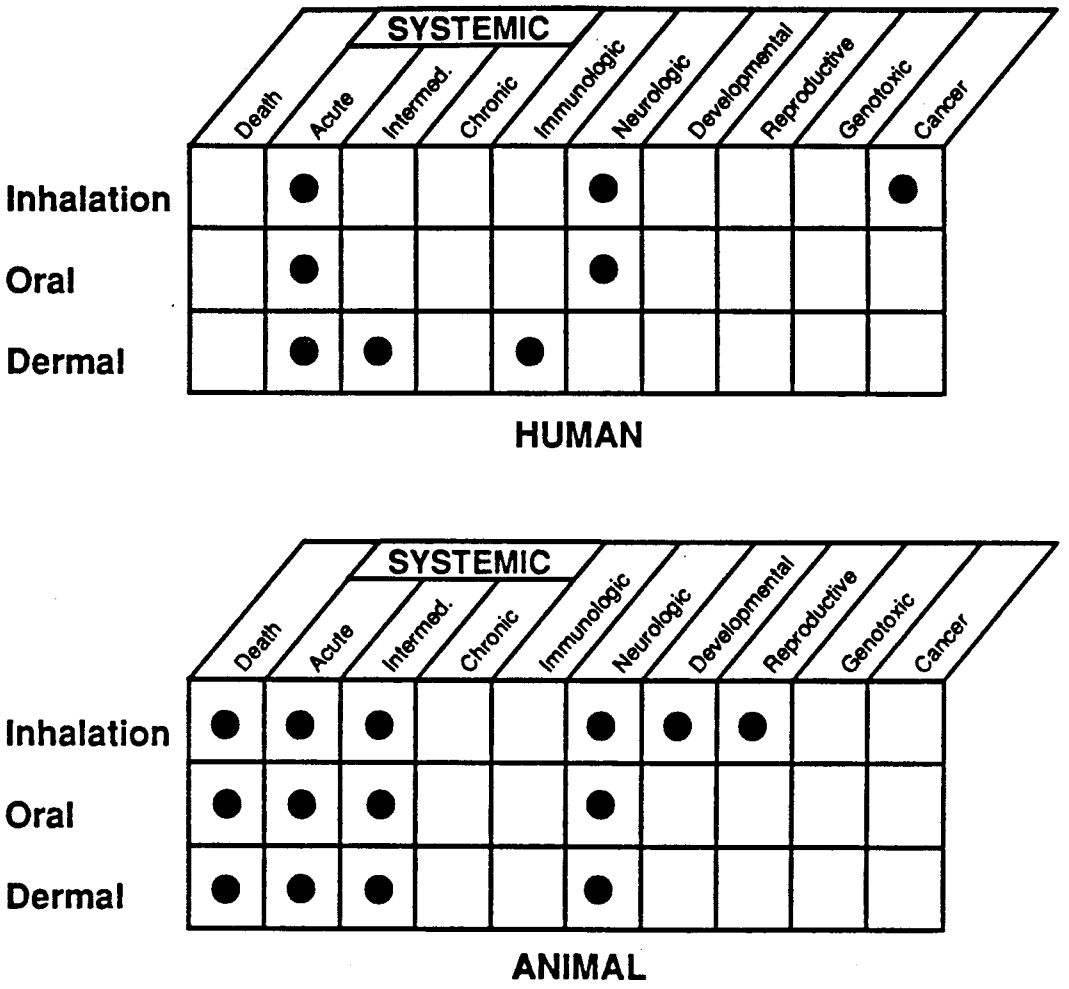
The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that, if met, would reduce or eliminate the uncertainties of human health assessment. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

2.9.1 Existing Information on Health Effects of 2-Butanone

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to 2-butanone are summarized in Figure 2-4. The purpose of this figure is to illustrate the existing information concerning the health effects of 2-butanone. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not imply anything about the quality of the study or studies. Gaps in this figure should not be interpreted as "data needs" information.

Studies regarding the adverse health effects of exposure to 2-butanone in humans is limited (Figure 2-4). No reports exist regarding death in humans

FIGURE 2-4. Existing Information on Health Effects of 2-Butanone



● Existing Studies

following exposure to 2-butanone by any route. Existing information regarding systemic effects of 2-butanone exposure have come primarily from two clinical reports involving accidental poisoning: one by oral exposure and one by inhalation (Berg 1971; Kopelman and Kalfayan 1983). A clinical study reported contact urticaria triggered by dermal -exposure to 2 butanone in a 48-year-old male painter (Varigos and Nurse 1986). Intermediate dermal exposure to 2-butanone had no effect on humans (Wahlberg 1984). Acute inhalation studies in humans showed no adverse neurological effects (Dick et al. 1984, 1988, 1989). However, 2-butanone produced nose, throat, and eye irritation in humans (Nelson et al. 1943). Epidemiological studies showed no clear relationship between occupational exposure to 2-butanone and the development of neoplasms (Alderson and Rattan 1980; Wen et al. 1985).

Animal studies regarding death after acute and intermediate exposure to 2-butanone by inhalation, oral, dermal, and other routes are available. Several studies are also available that show that acute and intermediate inhalation exposures have minimal or no systemic effects. Minimal effects were limited to small increases in organ weight (Cavender et al. 1983; Toftgard et al. 1981). Inhalation, oral, and dermal studies showed that 2-butanone is minimally neurotoxic in most species. Guinea pigs exposed acutely to high concentrations (10,000 ppm) developed incoordination and narcosis (Patty et al 1935). Juvenile baboons exposed to 100 ppm 2 butanone also appeared to show early signs of incoordination and narcosis in a complex discriminant neurobehavioral test (Geller et al. 1979). Histological examination of reproductive organs of male and female rats exposed by inhalation to 5,000 ppm revealed no effects (Cavender and Casey 1981; Cavender et al. 1983). Studies of acute oral exposure showed that 2-butanone caused renal tubular necrosis (Brown and Hewitt 1984; Hewitt et al. 1983) and induced hepatic microsomal enzymes (Brady et al. 1989; Raunio et al. 1990, Robertson et al. 1989; Traiger et al. 1989). One intermediate oral exposure showed that 2-butanone was not neurotoxic (Ralston et al, 1985). Acute and intermediate dermal exposures to 2-butanone were mildly irritating to the skin of rabbits, rats, and guinea pigs (Hazleton Laboratories 1963a; Wahlberg 1984). No reports of systemic toxicity are available for dermal exposure. Several studies demonstrating that 2-butanone is moderately irritating to the eyes of rabbits are available. 2-Butanone was fetotoxic, causing delayed development in fetuses of rats (Deacon et al. 1981; Schwetz et al. 1974) and mice (Mast et al. 1989) exposed by inhalation.

Several studies are available regarding 2-butanone potentiation of n-hexane and methyl-n-butyl ketone neurotoxicity and 2-butanone potentiation of haloalkane hepatotoxicity.

2.9.2 Data Needs

Acute-Duration Exposure. Several studies are available that report the results of acute duration exposure to 2-butanone by inhalation, oral, and dermal routes in both humans and animals. In acute inhalation studies, an

LC_{50} value in rats (LaBelle and Brieger 1955) and other exposures that caused death in rats (Klemisch 1988; Smyth et al. 1962), mice (LaBelle and Brieger 1955), and guinea pigs (Patty et al. 1935) were identified. The target organs identified in guinea pigs after acute inhalation exposure to high concentrations of 2-butanone are the respiratory system, liver, kidney, eye, and central nervous system Patty et al. 1935. Slight neurotoxicity was also seen in mice and baboons exposed to low concentrations (DeCeaurriz et al. 1983; Geller et al. 1979). Narcosis and incoordination were observed in guinea pigs exposed acutely to high concentrations of 2-butanone (Patty et al. 1935). An acute-duration inhalation MRL was not derived because target organs of rats and mice have not been sufficiently investigated. Although target organs of acute inhalation exposure of rats and mice have not been sufficiently investigated, intermediate duration studies indicate that effects on the liver, kidney, respiratory system, and nervous system of rats are minimal. In acute oral studies, LD_{50} values for rats (Kimura et al. 1971; Smyth et al. 1962) and mice (Tanii et al. 1986) were available, and the kidney was identified as a target (Brown and Hewitt 1984). No acute oral MRL was derived because target organs have not been sufficiently investigated. Furthermore, inhalation studies indicate that 2-butanone is a developmental toxicant, but developmental effects after oral exposure were not studied. Acute dermal studies have shown that 2-butanone is a skin and eye irritant in rabbits (Hazleton Laboratories 1963a, 1963b) and guinea pigs (Anderson et al. 1986; Wahlberg 1984), but the systemic toxicity of acute dermal exposure has not been investigated. The available pharmacokinetic data are not sufficient to predict whether target organs would be similar by the various routes of exposure. Acute exposure of humans near a toxic waste site to 2-butanone alone would probably not have serious clinical consequences. 2-Butanone is detectable by humans at concentrations far below its OSHA and NIOSH permissible levels because of its odor. Further acute exposure studies with 2-butanone alone would probably not be useful. In contrast, further acute exposure studies by oral, inhalation, and dermal routes of 2-butanone combined with such hepatotoxins as chloroform and carbon tetrachloride, or with such neurotoxins as n-hexane and methyl-n-butyl ketone would yield valuable information on the potentiation of the hepatotoxicity and neurotoxicity, resperhively, of the other-chemicals by 2-butanone. These chemicals are often found together in formulations used occupationally and they might be stored together at toxic waste sites. Therefore, workers and populations surrounding hazardous waste sites might be exposed to these substances for acute durations.

Intermediate-Duration Exposure. A comprehensive 90-day inhalation study in rats showed that 2-butanone did not have adverse effects in the respiratory, cardiovascular, gastrointestinal, musculoskeletal, hematological, hepatic, renal, or dermal/ocular systems (Cavender and Casey 1981; Cavender et al. 1983). The most serious effect was slightly increased liver weight at the highest concentration tested, 5,000 ppm. Occupational exposures to concentrations this high are unlikely since humans find 350 ppm 2-butanone intolerable (Nelson et al. 1943). No signs of neurotoxicity, either clinical

or histological, were observed in several studies of intermediate exposures to high concentrations of 2-butanone up to 6,000 ppm (Altenkirch et al. 1978a, 1978b; Cavender and Casey 1981; Cavender et al. 1983). Therefore, most organs and tissues in humans probably would not be adversely affected by intermediate 2-butanone exposures either occupationally or near toxic waste sites. An intermediate duration inhalation MRL was not derived because nose and throat irritation occurred in humans at acute inhalation exposure levels lower than the NOAEL values for intermediate duration inhalation exposure in animals. No intermediate oral or dermal studies investigated the systemic toxicity of 2-butanone by these routes, and the available pharmacokinetic data are not. Sufficient to predict whether target organs would be similar by the various routes of exposure. 2-Butanone has been detected in air, water, food, and soil (see Section 5.4); therefore, exposures by the inhalation, oral, and dermal routes are possible. From a public health perspective, exposure to solvent mixtures is more likely than exposure to a single pure chemical. Therefore, intermediate exposure studies of 2-butanone mixed with other solvents (hexacarbons and haloalkanes), the toxicity of which is potentiated by 2-butanone, would provide valuable information on neurotoxicity and systemic toxicity. This information is important since these chemicals are often found together in solvents used occupationally, and they might be stored together at hazardous waste sites where surrounding populations could be exposed for intermediate durations.

Chronic-Duration Exposure and Cancer. No studies were located regarding the health effects of chronic exposure to 2-butanone by any route in humans or animals, but acute and intermediate duration inhalation studies indicated that by itself, 2-butanone is minimally toxic. Pharmacokinetic data are insufficient to predict the possible target organs of chronic exposure by any route. Since 2-butanone has been detected in air, water, food, and soil (see Section 5.4), exposures by the inhalation, oral, and dermal routes are possible. 2-Butanone is often found in formulations with other chemicals, such as chloroform, carbon tetrachloride, n-hexane, and methyl-n-butyl ketone, the toxicities of which -2-butanone potentiates. These chemicals may be stored together at hazardous waste sites. Chronic inhalation, oral, and dermal studies in which animals are administered these chemicals in combination with 2-butanone may provide dose-response information for the potentiation of the neurotoxicity and hepatotoxicity of these chemicals by 2-butanone. This information is important because there are populations surrounding hazardous waste sites that might by exposed to these chemicals for similar durations.

Although no cancer bioassays were available, preliminary epidemiological studies suggest that occupational exposure to 2-butanone does not increase the development of neoplasms. Furthermore, 2-butanone was not genotoxic, either with or without metabolic activation, in several microorganisms and cultured mammalian cells (O'Donoghue et al. 1988; Thorpe 1982). Furthermore, no induction of micronuclei was found in the erythrocytes of hamsters (Basler 1986) or mice (O'Donoghue et al. 1988) after intraperitoneal injection with

2-butanone. On the basis of this information, 2-butanone does not appear to be carcinogenic.

Genotoxicity. No induction of micronuclei was found in the erythrocytes of hamsters (Basler 1986) or mice (O'Donoghue et al. 1988) after intraperitoneal injection with 2-butanone. A comprehensive battery of in vitro tests showed that 2-butanone was not mutagenic in two prokaryotic organisms and two eukaryotic organisms, did not transform mammalian cells in culture, and did not induce unscheduled DNA synthesis in rat primary hepatocytes (O'Donoghue et al. 1988; Thorpe 1982). 2-Butanone did not cause gene mutations in S. cerevisiae (Thorpe 1982), but caused mitotic chromosome loss (Whittaker et al. 1990; Zimmermann et al. 1989) and aneuploidy in S. cerevisiae (Mayer and Goin 1987) at high concentrations. The positive induction of chromosome loss in the yeast cells was enhanced by coexposure to 2-butanone, ethyl acetate, and propionitrile (Zimmermann et al. 1989). The positive induction of aneuploidy was enhanced by coexposure to 2-butanone and nocodazole (Mayer and Goin 1987). Although 2-butanone contains an electrophilic center at the carbonyl carbon, further testing for genotoxicity does not seem warranted, except in combination with other solvents.

Reproductive Toxicity. No studies were located regarding effects on reproductive capacity or reproductive organs and tissues in humans following exposure to 2-butanone. The authors of a health hazard evaluation report for NIOSH concluded that a perceived increase in the number of spontaneous abortions among female workers believed to result from exposure to 2-butanone and several other volatile chemicals at a shoe factory was not related to exposure (Tharr et al. 1982). No histopathological lesions were found in male or female reproductive organs of rats exposed to 5,000 ppm 2-butanone for 90 days (Cavender and Casey 1981; Cavender et al. 1983), but reproductive function was not assessed. Further studies of the reproductive function of 2-butanone by all durations and routes would provide valuable information particularly if the studies include histological examination of the organs and tissues of the reproductive. system. If reproductive organs were identified as targets of 2-butanone toxicity, single or multigeneration reproductive studies probably would be warranted. Since 2-butanone potentiates the neurotoxicity or hepatotoxicity of certain chemicals, it would be valuable to investigate the reproductive effects of mixed solvent exposures that include 2-butanone. This investigation would be useful because 2-butanone is often found in mixtures of other solvents in occupational settings, and these mixtures may be found together at or near hazardous waste sites.

Developmental Toxicity. Information regarding developmental toxicity of 2-butanone in humans was not located. 2-Butanone was slightly fetotoxic in rats (Deacon et al. 1981; Schwetz et al. 1979) and mice (Mast et al. 1989)following inhalation exposure of pregnant rats and mice to 3,000 ppm. The fetotoxicity was related to delayed development. Furthermore, five of eight pregnant rats exposed continuously to 800 ppm throughout gestation failed to deliver litters (Stoltenburg-Didinger et al. 1990). In addition,

developmental effects were more pronounced in pups born to rat dams exposed to a mixture of n-hexane and 2-butanone than in pups born to dams exposed to n-hexane alone (Stoltenburg-Didinger et al. 1990). This study, however, was very poorly reported, with very little information provided on exposure to 2-butanone alone. No developmental or distribution studies have been conducted by the oral route, but there is no reason to believe that 2-butanone or its metabolites could not cross the placenta after administration by the oral route. Therefore, it is likely that orally administered 2-butanone would be fetotoxic in these species. Determination of the doses needed to produce the fetotoxicity by the oral route would provide valuable information. Since 2-butanone potentiates the neurotoxicity or hepatotoxicity of certain chemicals, it would be valuable to further investigage the developmental effects of mixed solvent exposures that include 2-butanone. Such a study would be useful because 2-butanone is often found in mixtures with other solvents in occupational settings, and these mixtures may be fould at or near hazardous waste sites.

Immunotoxicity. No studies were located regarding immunotoxicity after oral exposure to 2-butanone. A clinical report of contact urticaria in a 47-year-old painter exposed occupationally to 2-butanone (Varigos and Nurse 1986) suggests that skin sensitivity requires more study. Altenkirch et al. (1978a) reported that 19/19 rats died suddenly of pathologically confirmed bronchopneumonia after 7 weeks of inhalation exposure to 6,000 ppm 2-butanone. 2-butanone may weaken the immune system, thus predisposing humans and animals to infection. No histopathological lesions were found in the thymus, lymph nodes, spleen, or bone marrow of rats exposed to 5,000 ppm or less of 2-butanone for 90 days (Cavender and Casey 1981; Cavender et al. 1983), but tests for immune function were not performed. Therefore, a study of the effects of 2-butanone on immune function (thymus, lymph nodes, peripheral blood lymphocytes, etc.)would provide valuable information regarding the immunotoxicity of 2-butanone.

Neurotoxicity. 2-butanone was not neurotoxic at a concentration of 200 ppm in several acute inhalation exposure studies in humans (Dick et al. 1984, 1988, 1989). Neurobehavioral effects have been observed in mice (1,602 ppm))DeCeaurriz et al. 1983) and baboons (100 ppm) (Geller et al. 1979) exposed acutely by inhalation. Guinea pigs displayed narcosis and incoordination after acute inhalation exposure to high concentrations (Patty et al. 1935). Clinical signs of neurotoxicity were also observed in rats treated acutely by gavage with a high dose of 2-butanone (Stillmeadow Inc. 1978). However, 2-butanone is not generally regarded as being highly neurotoxic when administred alone. In acute and intermediate exposure studies in animals, it markedly potentiated the neurotoxicity of n-hexane and methyl-n-butyl ketone both in humans and animals. A comprehensive study of acute, intermdiate, and chronic exposures to mixtures of 2-butanone, n-hexane, and methyl-n-butyl ketone by inhalation, oral, and dermal routes will provide valuable information regarding the neurotoxicity of these compounds. Such a study would be particularly valuable because 2-butanone is

often found occupationally in mixtures containing n-hexane and methyl-n-butyl ketone, and these chemicals would probably be found together at hazardous waste sites.

Epidemiological and Human Dosimetry Studies. One study with humans etermined that inhalation exposure to 100 ppm for 15 minutes was irritating to the nose and throat, and exposure to 350 ppm was intolerable (Nelson et al. 1943). In three separate studies, volunteers exposed to 200 ppm had no neurobehavioral effects (Dick et al. 1984, 1988, 1989). Two epidemiological studies of chemical company workers exposed to 2-butanone showed inconclusive results regarding increased risk of cancer (Alderson and Rattan 1980; Wen et al. 1985). No epidemiological studies regarding other health effects of 2-butanone exposure were located. Therefore, valuable epidemiological information could be obtained from further studies of cancer and other health effects, particularly neurotoxicity and reproductive and developmental toxicity.

Biomarkers of Exposure and Effect. The only known biomarkers of 2-butanone exposure are blood, breath, and urinary concentrations of 2-butanone and its metabolites (Brown et al. 1986; Brugnone et al. 1983; Ghittori et al. 1987; Miyasaka et al. 1982). 2-Butanone is rapidly cleared from the body, and existing studies show that accumulation of 2-butanone in tissues does not occur to a significant extent. Furthermore, 2-butanone alone is relatively free of adverse health effects. Therefore, development of biomarkers of exposure to a battery of solvents often used occupationally in combination with 2-butanone would be more valuable than development of biomarkers for 2-butanone alone.

2-Butanone exposure has no specific effects that can be used as biomarkers for exposure by any route or for any duration of exposure.

Absorption, Distribution, Metabolism, and Excretion. 2-Butanone is absorbed by inhalation (Liira et al. 1988a, 1988b, 1990, 1991) and oral exposure (Brown and Hewitt 1984; Dietz and Traiger 1979; Dietz et al. 1981; Hewitt et al. 1983; Sakata et al. 1989). Net retention of inhaled 2-butanone is approximately 50% in humans (Liira et al. 1988a, 1988b). Studies of absorption after dermal exposure would provide valuable information on this occupationally significant route of entry. Available data regarding the relative rates or extent of absorption, metabolism, distribution, and excretion by the three routes of exposure are not sufficient to draw meaningful conclusions. 2-Butanone is equally soluble in all tissues and organs measured (Perbellini et al. 1984). Therefore, 2-butanone is probably evenly distributed throughout the body. The primary route of excretion appears to be the lungs. The metabolic pathways for 2-butanone have been thoroughly studied in rats (Dietz and Traiger 1979; Dietz et al. 1981) and guinea pigs (DiVincenzo et al. 1976). Similar metabolites have been identified in humans (Liira et al. 1988a, 1988b; Miyasaka et al. 1982). In rats, 30% of an oral dose of 2-butanone was converted to 2,3-butanediol (Dietz

et al. 1981). Potentiation of the neurotoxicity of ethanol, n-hexane, and methyl-n-butyl ketone and the hepatotoxicity of haloalkanes by 2-butanone may involve interactions in the biotransformation of these compounds (Brady et al. 1989; Cunningham et al. 1989; Raunio et al. 1990; Robertson et al. 1989; Traiger et al. 1989). Further studies regarding the interaction of hexacarbons, haloalkanes, and 2-butanone at the metabolic level may provide valuable information.

Comparative Toxicokinetics. Available human data show that 2 butanone is metabolized primarily to 2,3-butanediol and 3-hydroxy-2-butanone, but the extent of metabolism appears to be small (Liira et al. 1988a, 1988b). In an occupational exposure study of 2-butanone, only 3-hydroxy-2-butanone was observed (Brugnone et al. 1983). In rats and guinea pigs, a third metabolite, 2-butanol, was observed (Dietz et al. 1981; DiVincenzo et al. 1976). About 30% of an oral dose of 2-butanone in rats later appeared in plasma as 2,3-butanediol (Dietz et al. 1981). 2-Butanol is also a product of 2-butanone metabolism in humans (Liira et al. 1990). 2-Butanone potentiates the neurotoxicity of n-hexane and methyl-n-butyl ketone and the hepatotoxicity of haloalkanes. The 2-butanone metabolite, 2,3-butanediol, may be more efficacious for potentiating the hepatotoxicity of the haloalkanes than 2-butanone. Therefore, valuable information would be gained by toxicokinetic studies of 2-butanone and its metabolites as they pertain to the toxicity of the hexacarbons and haloalkanes.

Mitigation of Effects. 2-Butanone by itself has a low order of systemic toxicity. However, exposure to 2-butanone with other solvents, which is more likely in occupational and environmental settings than is exposure to 2-butanone alone, results in a potentiation of the neurotoxicity and hepatotoxicity of the other solvents. Further studies that investigate the mechanism by which 2-butanone potentiates the toxicity of other ketones, n-hexane, chloroform, and carbon tetrachloride would be useful in planning research aimed to develop agents that would interfere with the mechanism, thereby mitigating the potentiation.

2.9.3 On-going Studies

No information regarding current studies of the health effects of 2-butanone was located.

3. CHEMICAL AND PHYSICAL INFORMATION

3.1 CHEMICAL IDENTITY

Data pertaining to the chemical identity of 2-butanone are listed in Table 3-1.

3.2 PHYSICAL AND CHEMICAL PROPERTIES

The physical and chemical properties of 2-butanone are presented in Table 3-2.

3. CHEMICAL AND PHYSICAL INFORMATION

TABLE 3-1. Chemical Identity of 2-Butanone

Characteristic	Information	Reference
Chemical name	2-Butanone	CAS 1989
Synonyms	Methyl ethyl ketone; MEK; ethyl methyl ketone; methyl acetone; and others	CAS 1989; SANSS 1989; Chemline 1989
Trade name(s)	Meetco	OHM/TADS 1989
Chemical formula	C_4H_8O	CAS 1989
Chemical structure	$CH_3-\overset{\overset{\displaystyle O}{\|}}{C}-CH_2-CH_3$	
Identification numbers:		
CAS registry	78-93-3	CAS 1989
NIOSH RTECS	EL6475000	HSDB 1989
EPA hazardous waste	U159	HSDB 1989
OHM/TADS	7216796	Chemline 1989
DOT/UN/NA/IMCO	UN1193, UN1232	Chemline 1989
HSDB	99	HSDB 1989
NCI	No data	

CAS = Chemical Abstracts Service
EPA = Environmental Protection Agency
DOT/UN/NA/IMCOP = Department of Transportation/United Nations/North America/ International Maritime Consultive Organization
HSDB = Hazardous Substance Data Bank
NCI = National Cancer Institute
NIOSH = National Institute for Occupational Safety and Health
OHM/TADS = Oil and Hazardous Materials Technical Assistance Data Base
RTECS = Registry of Toxic Effects of Chemical Substances
SANSS = Structure and Nomenclature Search System

TABLE 3-2. Physical and Chemical Properties of 2-Butanone

Property	Information	Reference
Molecular weight	72.11	Weast et al. 1988
Color	Colorless	Sax and Lewis 1987
Physical state	Liquid	Sax and Lewis 1987
Melting point	-86.3°C	Weast et al. 1988
Boiling point	79.6°C	Weast et al. 1988
Density (liquid) at 20°C	0.8054	Weast et al. 1988
Odor	Acetone-like	Sax and Lewis 1987
Odor threshold		
Water	8.4 ppm	Amoore and Hautala 1983
Air	5.4 ppm	Amoore and Hautala 1983
Solubility		
Water at 25°C	136,000 mg/L	Tewari et al. 1982
Organic solvents	Benzene, alcohol, ether, oils, most organic solvents	Sax and Lewis 1987; Neier and Strehlke 1985
Partition coefficient		
Log octanol/water	0.29	Hansch and Leo 1985
Log K_{oc}	0.55	Roy and Griffin 1985
Vapor pressure at 25°C	90.6 mmHg	Riddick et al. 1986
Henry's law constant at 25°C	5.77×10^{-5} atm m^3/mol	Rathburn and Tai 1987
Autoignition temperature	515°C	Sax and Louis 1987
Flashpoint		
Closed cup	-2°C	Riddick et al. 1986
Open cup	1°C	Riddick et al. 1986
Flammability limits in air	2-10%	Sax and Lewis 1987
Conversion factors		
ppm (v/v) to mg/m^3 in air (20°C)	1 ppm = 2.93 mg/m^3	
mg/m^3 to ppm (v/v) in air (20°C)	1 mg/m^3 = 0.341 ppm	
Bioconcentration factor	0.98 (calculated from K_{ow})	Lyman et al. 1982
Explosive limits	No data	

4. PRODUCTION, IMPORT, USE, AND DISPOSAL

4.1 PRODUCTION

According to the most recent edition of U.S. International Trade Commission (USITC 1989), 482,028,000 pounds of 2-butanone were produced in the United States in 1988. The production volume for 1986 and 1987 was 600,440,000 and 671,859,000 pounds, respectively (USITC 1987, 1988). Total U.S. capacity for 1989 has been estimated at 622 million pounds (SRI 1989). Production of 2-butanone has been flat in the past decade, but it is expected to grow at 2%-3% through 1991 (Chemical Marketing Reporter 2987). Current manufacturers of 2-butanone are included in Table 4-1. According to the Toxic Release Inventory (TRI 1989), 2,218 facilities manufacture or process 2-butanone. These facilities had a maximum amount of 2-butanone on site of approximately 1,670,000,000 pounds in 1987. These data are presented in Table 4-2. The quality of the data must be viewed with caution since the 1987 data represent first-time, incomplete reporting by these facilities. Not all facilities that should have reported have done so.

2-Butanone is produced on a commercial scale by one of two processes. The vapor-phase dehydrogenation of set-butanol.(2-butanol), itself obtained from the hydrolysis of butene, accounts for 88% 2-butanone production (Neier and Strehlke 1985; Papa and Sherman 1981). In the other commercially significant process, 2-butanone is obtained as a byproduct of acetic acid production. In this methodology, liquified butane is subjected to catalytic oxidation.

4.2 IMPORT/EXPORT

Approximately 16% of the total U.S. production of 2-butanone is exported to other countries (Chemical Marketing Reporter 1987). Imports into the United States amounted to about 52 million pounds in 1986.

4.3 USE

2-Butanone exhibits outstanding solvent properties, and combined with its low cost, it is often the choice solvent for various coating systems (Neier and Strehlke 1985; Papa and Sherman 1981). Uses of 2-butanone can be broken down into the following categories: coatings solvent, 50%; adhesives, 13%; magnetic tapes, 8%; lube oil dewaxing, 4%; printing inks, 3%; exports, 16%; and miscellaneous, 6% (Chemical Marketing Reporter 1987). Examples of specific applications include its use as a solvent for nitrocellulose, lacquers, rubber cement, printing inks, paint removers, vinyl films, resins, rosins, polystyrene, chlorinated rubber, polyurethane, acrylic coatings, and cleaning solutions (Neier and Strehlke 1985; Papa and Sherman 1981; Sax and Lewis 1987). 2-Butanone is used in the production of synthetic leathers, transparent paper, and aluminum foil. It is also used in the degreasing of metals, as an extraction solvent, in dewaxing applications, and as a solvent for the production of smokeless powders.

4. PRODUCTION, IMPORT, USE, AND DISPOSAL

TABLE 4-1. Current Manufacturers of 2-Butanone[a]

Company	Location
ARCO Chemical Co.	Channelview, TX
Exxon Corporation	Baton Rouge, LA
Hoechst Celanese Corp.	Pampa, TX
Shell Oil Company	Norco, LA
Union Carbide Corp	No data

[a]Derived from SRI 1989; USITC 1989

TABLE 4-2. Facilities That Manufacture or Process 2-Butanone[a]

State[b]	No. of facilities	Range of maximum amounts on site in thousands of pounds[c]	Activities and uses[d]
AL	29 (2)[e]	0.1-999	2, 3, 8, 9, 10, 11, 12, 13
AR	41 (2)[e]	0-999	1, 2, 3, 7, 8, 9, 11, 12, 13
AZ	21 (1)[e]	0.1-99	2, 7, 8, 11, 12, 13
CA	193 (12)[e]	0-9,999	1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 13
CO	14 (2)[e]	0.1-99	8, 9, 11, 12, 13
CT	37 (1)[e]	0-999	2, 8, 9, 10, 11, 12, 13
DE	10	1-999	3, 8, 11, 12, 13
FL	29 (3)[e]	0-999	2, 3, 4, 7, 8, 10, 11, 12, 13
GA	58 (2)[e]	0-999	2, 3, 4, 7, 8, 9, 10, 11, 12, 13
IA	30 (1)[e]	0-49,999	2, 8, 9, 10, 11, 12, 13
ID	1	1-9	11, 13
IL	133 (10)[e]	0.1-99,999	2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 13
IN	104 (7)[e]	0-999	2, 3, 5, 7, 8, 9, 10, 11, 12, 13
KS	26 (1)[e]	0-999	2, 3, 4, 5, 7, 8, 9, 11, 12, 13
KY	30 (2)[e]	0-9,999	1, 3, 8, 10, 11, 12, 13
LA	29 (1)[e]	0.1-49,999	1, 2, 3, 4, 7, 8, 9, 11, 12, 13
MA	60 (5)[e]	0-999	1, 2, 3, 4, 7, 8, 9, 10, 11, 12, 13
MD	21	0-99	3, 8, 9, 11, 12, 13
ME	5	0.1-99	11, 12, 13
MI	119 (8)[e]	0-999	2, 3, 7, 8, 9, 10, 11, 12, 13
MN	35 (2)[e]	0-9,999	2, 3, 4, 6, 8, 9, 10, 11, 12, 13

TABLE 4-2 (Continued)

State[b]	No. of facil-ities	Range of maximum amounts on site in thousands of pounds[c]	Activities and uses[d]
MO	72 (4)[e]	0-499,999	2, 3, 7, 8, 9, 10, 11, 12, 13
MS	21 (1)[e]	0-999	2, 8, 9, 11, 12, 13
MT	1	1-9	8, 11
NC	133 (13)[e]	0-999	3, 5, 7, 8, 9, 10, 11, 12, 13
ND	3	0.1-9	11, 12, 13
NE	14 (1)[e]	0.1-99	5, 7, 8, 9, 11, 12, 13
NH	23 (1)[e]	0-999	2, 3, 7, 8, 9, 10, 11, 12, 13
NJ	103 (7)[e]	0-49,999	1, 2, 3, 4, 7, 8, 9, 10, 11, 12, 13
NM	1	1-9	12, 13
NV	4 (1)[e]	0.1-99	9, 12
NY	85 (15)[e]	0.1-49,999	1, 2, 3, 7, 8, 9, 10, 11, 12, 13
OH	174 (10)[e]	0-49,999	2, 3, 7, 8, 9, 10, 11, 12, 13
OK	17	0.1-999	2, 4, 8, 9, 11, 12, 13
OR	13 (1)[e]	0.1-99	8, 9, 10, 11, 12, 13
PA	109 (5)[e]	0-499,999	1, 2, 3, 4, 7, 8, 9, 10, 11, 12, 13
PR	9 (2)[e]	0-999	8, 9, 12
RI	13 (1)[e]	1-99,999	3, 4, 8, 11, 12, 13
SC	36 (4)[e]	0.1-49,999	2, 3, 4, 7, 8, 9, 11, 12, 13
SD	5	1-99	8, 11, 13
TN	60 (6)[e]	0-9,999	2, 3, 5, 7, 8, 9, 11, 12, 13
TX	107 (2)[e]	0-49,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13

TABLE 4-2 (Continued)

State[b]	No. of facil-ities	Range of maximum amounts on site in thousands of pounds[c]	Activities and uses[d]
UT	10	0-99	8, 9, 11, 12, 13
VA	64 (1)[e]	0-49,999	3, 7, 8, 9, 10, 11, 12, 13
VT	4	1-9	8, 11, 12, 13
WA	32 (5)[e]	1-999	8, 10, 11, 12, 13
WI	71 (3)[e]	0-999	1, 2, 3, 4, 7, 8, 9, 10, 11, 12, 13
WV	8 (3)[e]	1-9,999	1, 3, 5, 6, 10, 11, 12, 13
WY	1	10-99	7

[a]TRI 1989
[b]Post office state abbreviations
[c]Data in TRI are maximum amounts on site at each facility.
[d]Activities/Uses:

1. produce
2. import
3. for on-site use/processing
4. for sale/distribution
5. as a byproduct
6. as an impurity
7. as a reactant
8. as a formulation component
9. as an article component
10. for repackaging only
11. as a chemical processing aid
12. as a manufacturing aid
13. ancillary or other use

[e]Number of facilities reporting "no data" regarding maximum amount of the substance on site.

4.4 DISPOSAL

Dilute solutions of 2-butanone can be discharged directly into sewage treatment facilities, sprayed into incinerators, or burned in paper packaging (OHM/TADS 1989). It can be destroyed in fluidized-bed incinerators, rotary kiln incinerators, or liquid injection incinerators using short residence times of a few seconds for either liquids or gases, and longer residence times for contaminated solids, if applicable (HSDB 1989). 2-Butanone has been reported to be amenable to biological degradation in sewage treatment plants (Babeu and Vaishnav 1987; Bridie et al. 1979; Gaudy et al. 1963.; Price et al. 1974; Urano and Kato 1986; Vaishnav et al. 1987; Young et al. 1968). No data are available regarding the amount disposed by each of these methods, nor is any information available regarding the trends in the disposal of 2-butanone.

5. POTENTIAL FOR HUMAN EXPOSURE

5.1 OVERVIEW

2-Butanone may be released to the atmosphere in fugitive emissions during its production, transport, and use. It is widely used in coating systems where its volatilization to the atmosphere is an intended outcome of its use. In urban areas, it can exist in the atmosphere as a result of automobile exhaust, the decomposition of other organic compounds, and from natural sources.

The release of 2-butanone to water or soil is not well documented. Release of 2-butanone to surface water may occur via industrial waste water emissions. 2-Butanone may also be released to soil or water from a spill or other catastrophic event. The leachate of landfills and hazardous waste sites may result in 2-butanone contamination of soil and groundwater.

According to the SARA Section 313 TRI (1989), an estimated total of 149,678,423 pounds of 2-butanone was released to the environment in 1987 by facilities that manufacture or process this compound. Of this, 149,478,640 pounds were released to the atmosphere. The quality of the TRI data must be viewed with caution since the 1987 data represent first-time, incomplete reporting of estimated releases by these facilities. Not all sources of chemical wastes are included and not all facilities. Only certain types of facilities were required to report. This is not an exhaustive list. These data are presented in Table 5-1.

2-Butanone is expected to rapidly volatilize from surface water and moist or dry soils to the atmosphere, In the atmosphere, this compound is expected *to* exist predominantly in the vapor phase. Wet deposition may return 2-butanone to the earth's surface.

In soil, 2-butanone is expected to display very high mobility, and it has the potential to leach into groundwater. This characteristic also suggests that it does not significantly adsorb to sediment and suspended organic matter in surface waters. 2-Butanone is not expected to bioconcentrate in fish and aquatic organisms.

Although the degradation of 2-butanone in the environment is understood on a theoretical level, data are not available to quantify all conclusions. In the atmosphere, 2-butanone is expected to undergo a vapor-phase reaction with photochemically produced hydroxyl radicals; the half-life for this process is approximately 1 day. However, laboratory experiments have suggested that the atmospheric half-life of 2-butanone is much shorter.

In water, 2-butanone is expected to undergo microbial degradation under both aerobic and anaerobic conditions. Chemical oxidation, direct photolysis, and hydrolysis of 2-butanone under environmental conditions are not expected to occur to any significant extent. Data on the fate of 2-butanone in soil are not available.

TABLE 5-1. Releases to the Environment from Facilities That Manufacture or Process 2-Butanone[a]

State[c]	No. of facil-ities	Range of reported amounts released in thousands of pounds[b]						
		Air	Underground injection	Water	Land	Total Environment[d]	POTW[e] transfer	Off-site waste transfer
AL	29	0-4,000	0-0	0-0	0-1	0-4,000	0-1	0-113
AR	41	0-968	0-0	0-0	0-17	0-968	0-50	0-70
AZ	21	0-125	0-0	0-0	0-0	0-125	0-0	0-151
CA	193	0-690	0-0	0-0	0-1	0-690	0-24	0-822
CO	14	0-159	0-0	0-0	0-0	0-159	0-0	0-8
CT	37	0-123	0-0	0-0	0-0	0-123	0-0	0-57
DE	10	0-623	0-0	0-0	0-0	0-623	0-10	0-230
FL	29	0-88	0-0	0-0	0-0	0-88	0-7	0-51
GA	58	0-812	0-0	0-0	0-0	0-812	0-12	0-692
IA	30	0-1,201	0-0	0-0	0-0	0-1,201	0-0	0-76
ID	1	2-2	0-0	0-0	0-0	2-2	0-0	14-14
IL	133	0-440	0-0	0-0	0-0	0-440	0-11	0-839
IN	104	0-1,400	0-0	0-0	0-0	0-1,400	0-1	0-466
KS	26	0-777	0-0	0-1	0-0	0-777	0-0	0-130
KY	30	0-130	0-0	0-0	0-0	0-130	0-0	0-390
LA	29	0-745	0-5	0-0	0-0	0-746	0-0	0-39
MA	60	0-601	0-0	0-0	0-0	0-601	0-27	0-508
MD	21	0-714	0-0	0-0	0-0	0-714	0-0	0-242
ME	5	0-111	0-0	0-0	0-0	0-111	0-0	0-30
MI	119	0-1,300	0-0	0-0	0-1	0-1,301	0-15	0-294
MN	35	0-10,219	0-0	0-0	0-4	0-10,219	0-0	0-1,734
MO	72	0-1,000	0-0	0-1	0-0	0-1,000	0-5	0-302
MS	21	1-4,529	0-0	0-0	0-0	1-4,529	0-4	0-1,290
MT	1	8-8	0-0	0-0	0-0	8-8	0-0	14-14
NC	133	0-980	0-0	0-0	0-0	0-980	0-0	0-276
ND	3	2-15	0-0	0-0	0-0	2-15	0-0	0-14
NE	14	0-277	0-0	0-0	0-0	0-277	0-0	0-8
NH	23	0-146	0-0	0-0	0-0	0-146	0-0	0-350
NJ	103	0-678	0-0	0-2	0-0	0-678	0-56	0-277
NM	1	12-12	0-0	0-0	0-0	12-12	0-0	0-0
NV	4	0-26	0-0	0-0	0-0	0-26	0-0	0-17
NY	85	0-470	0-0	0-3	0-0	0-470	0-59	0-196
OH	174	0-1,890	0-0	0-0	0-1	0-1,890	0-43	0-890
OK	17	0-1,242	0-0	0-0	0-0	0-1,242	0-0	0-555
OR	13	2-492	0-0	0-0	0-0	2-492	0-4	0-12
PA	109	0-1,259	0-0	0-2	0-4	0-1,259	0-4	0-264
PR	9	0-230	0-0	0-0	0-0	0-230	0-0	0-0
RI	13	0-542	0-0	0-0	0-0	0-542	0-0	0-110
SC	36	0-668	0-0	0-1	0-0	0-668	0-0	0-172
SD	5	19-48	0-0	0-0	0-0	19-48	0-0	0-40
TN	60	0-504	0-0	0-1	0-1	0-504	0-1	0-166
TX	107	0-1,304	0-52	0-9	0-1	0-1,304	0-6	0-400

TABLE 5-1 (Continued)

State[c]	No. of facil-ities	Range of reported amounts released in thousands of pounds[b]						
		Air	Underground injection	Water	Land	Total Environment[d]	POTW[e] transfer	Off-site waste transfer
UT	10	0-48	0-0	0-0	0-0	0-48	0-0	0-15
VA	64	0-1,495	0-0	0-0	0-10	0-1,495	0-3	0-214
VT	4	1-55	0-0	0-0	0-0	1-55	0-0	1-9
WA	32	0-2,130	0-0	0-0	0-1	0-2,130	0-1	0-180
WI	71	0-890	0-0	0-39	0-0	0-890	0-91	0-720
WV	8	0-235	0-0	0-6	0-0	0-235	0-2	0-45
WY	1	83-83	0-0	0-0	0-0	83-83	0-0	0-0

[a]TRI 1989
[b]Data in TRI are maximum amounts released by each facility.
[c]Post office state abbreviation
[d]The sum of all releases of the chemical to air, land, water, and underground injection wells by a given facility.
[e]publicly owned treatment works

5. POTENTIAL FOR HUMAN EXPOSURE

Various data are available regarding the concentration of 2-butanone in environmental media. It has been qualitatively detected in U.S. drinking water supplies and as a naturally occurring constituent of foods. It has also been detected in the air.

EPA has identified 1,177 NPL sites. 2-Butanone has been found at 137 out of the sites evaluated for its presence. However, we do not know how many of the 1,177 NPL sites have been evaluated for this chemical. As more sites are evaluated by EPA, this number may change (View 1989). The frequency of these sites within the United States can be seen in Figure 5-l.

The general population is exposed to 2-butanone by drinking contaminated water or by the ingestion of food containing it. Members of the general population living near hazardous waste sites may be exposed to contaminated drinking water if their household water source is well water. The general population is also expected to be exposed to 2-butanone by inhalation, especially in urban areas. The use of commercial coatings containing 2-butanone also results in exposure by inhalation, and possibly by dermal contact as well. High levels of exposure may occur for members of the general population if these coatings are used in an enclosed, unventilated area. Occupational exposure to 2-butanone may occur by inhalation during the production, formulation, use, or transport of this compound.

5.2 RELEASES TO THE ENVIRONMENT

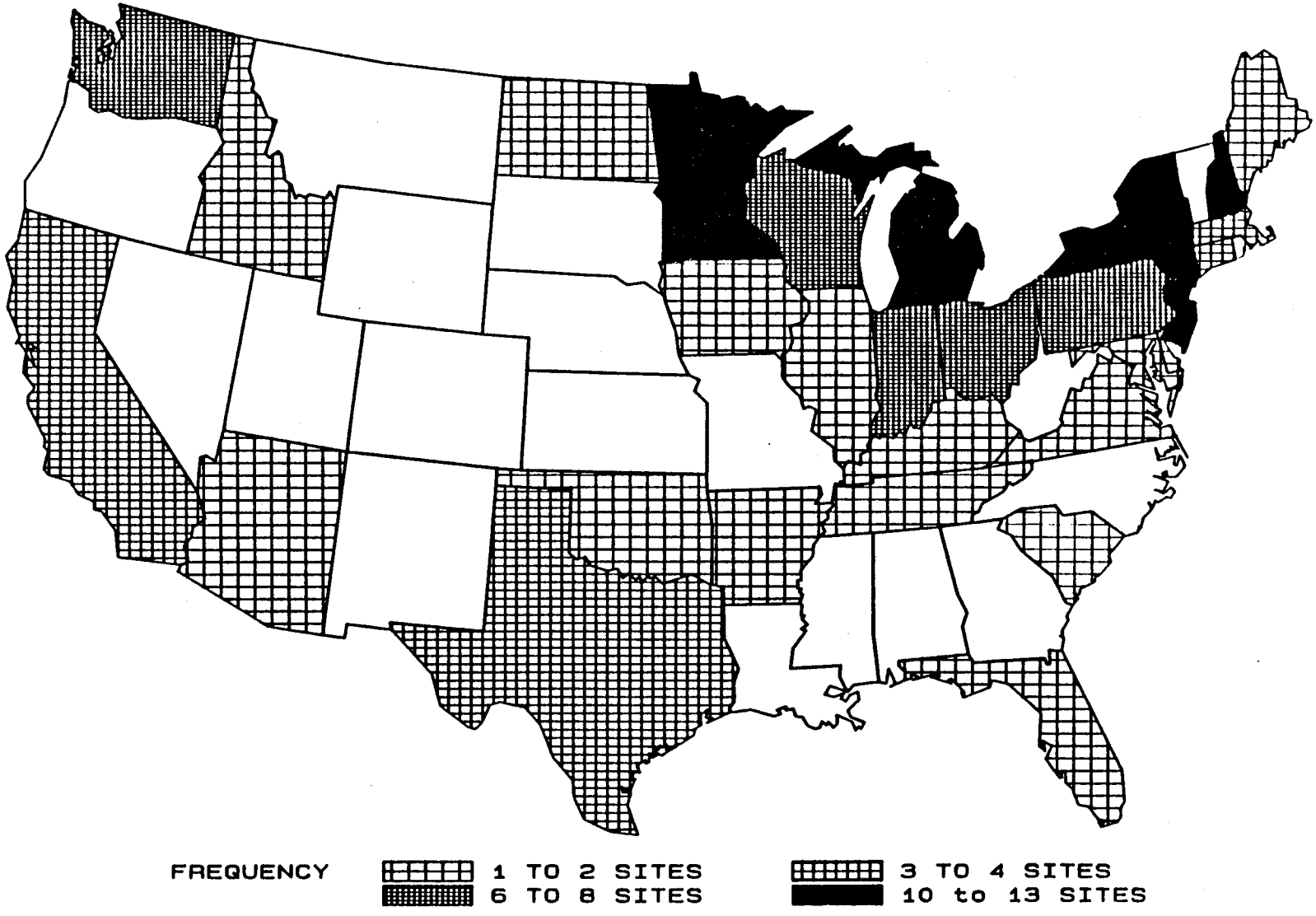
5.2.1 Air

2-Butanone may be emitted to the atmosphere during its production, formulation, storage, or use in commercial products. 2-Butanone may also be released to the atmosphere as a result of its use as a solvent in commercial products. It was identified as an emission from a variety of indoor building materials: latex caulk, particle board, latex paint, and polyurethane floor finish (Tichenor 1987; Tichenor and Mason 1988). Since 2-butanone is prevalent in adhesives and coatings (Papa and Sherman 1981), it may be released to the atmosphere during the curing of these products.

According to the SARA Section 313 TRI (1989), an estimated total of 149,478,640 pounds of 2-butanone was released to the atmosphere in 1987 by facilities that manufacture or process this compound. The quality of the TRI data must be viewed with caution since the 1987 data represent first-time, incomplete reporting of estimated releases by these facilities. Only certain types of facilities were required to report. This is not an exhaustive list.

2-Butanone is present in the exhaust of automobiles (Seizinger and Dimitriades 1972). In a Swedish study, 2-butanone was detected in automobile exhaust, although the ambient air levels measured in Stockholm did not correlate with these emissions (Jonsson et al. 1985). Thus, the prevalence of other sources is indicated, as the air levels of 2-butanone were higher than

FIGURE 5-1. FREQUENCY OF NPL SITES WITH 2-BUTANONE CONTAMINATION *



* Derived from View 1989

could be explained solely by automobile emissions. Other potential sources of 2-butanone in the atmosphere include the burning of polyethylene (Hodgkin et al. 1982) and the photochemical degradation of hydrocarbons (Grosjean 1982), especially those emitted from motor vehicles. 2-Butanone is also emitted to the atmosphere from such natural sources as European firs, junipers, cedars, cypress trees, and ferns (Isidorov et al. 1985) and ant secretions (Cammaerts et al. 1978).

5.2.2 Water

Limited data are available regarding the release of 2-butanone to surface and groundwaters. It has been detected in waste water effluents from commercial processes (Dunovant et al. 1986; Hawthorne and Sievers 1984; Jungclaus et al. 1978; Pellizzari et al. 1979). 2-Butanone may also be present in water from the microbial oxidation of butane (Phillips and Perry 1974). Its relatively high water solubility, 136,000 mg/L at 25°C (Tewari et al. 1982), suggests that wet deposition of atmospheric 2-butanone results in the contamination of surface water. Evidence for this comes from the fact that 2-butanone has been detected in rain water (Grosjean and Wright 1983).

According to the SARA Section 313 TRI (1989), an estimated total of 75,858 pounds of 2-butanone was released to water in 1987 by facilities that manufacture or process this compound, a very small amount compared to what is released to the atmosphere. The quality of the TRI data must be viewed with caution since the 1987 data represent first-time, incomplete reporting of estimated releases by these facilities. Only certain types of facilities were required to report. This is not an exhaustive list.

The contamination of groundwater with 2-butanone has occurred at hazardous waste sites (Francis et al. 1980; Sawhney and Kozloski 1984) and landfills (Sabel and Clark 1984) due to infiltration of contaminated leachate. 2-Butanone was detected in 180 groundwater samples from 357 hazardous waste sites monitored by the Contract Laboratory Program (CLP) at a geometric mean concentration of 302 ppb for the positive samples (CLPSD 1988). Note that the CLPSD includes data from both NPL and non-NPL sites. 2-Butanone is also likely to enter groundwater as a result of a spill to soil during a catastrophic event, such as a tanker spill (Halvorsen and Ohneck 1985).

2-Butanone may also enter water from natural sources. It has been detected in various species of macroalgae at concentrations as high as 2,600 ng/g (Whelan et al. 1982).

5.2.3 Soil

Limited data are available regarding the release of 2-butanone to soil. The presence of this compound in the groundwater at hazardous waste sites and landfills (Francis et al. 1980; Sabel and Clark 1984; Sawhney and Kozloski 1984) suggests that leachate at these facilities will be a source of

2-butanone release to soil. Wet deposition of atmospheric 2-butanone may also result in its contamination of soil. 2-Butanone may enter soil during a catastrophic event, such as a tanker spill (Halvorsen and Ohneck 1985). 2-Butanone has been found in 309 of 357 hazardous waste sites monitored by the CLP at a geometric mean concentration of 87 ppb (CLPSD 1988). Note that the CLPSD includes data from both NPL and non-NPL sites.

According to the SARA Section 313 TRI (1989), an estimated total of 48,675 pounds of 2-butanone was released to soil in 1987 by facilities that manufacture or process this compound; a very small amount compared to what is released to the atmosphere. The quality of the TRI data must be viewed with caution since the 1987 data represent first-time, incomplete reporting of estimated releases by these facilities. Only certain types of facilities were required to report. This is not an exhaustive list.

5.3 ENVIRONMENTAL FATE

5.3.1 Transport and Partitioning

In the atmosphere, 2-butanone is expected to exist predominantly in the vapor phase (Eisenreich et al. 1981; Riddick et al. 1986). This is consistent with experimental data, which demonstrated that the gas-phase concentration of 2-butanone in Los Angeles, California, was from 220 to 3,000 times greater than the particulate phase concentration (Grosjean 1982). The relatively high water solubility of 2-butanone, 136,000 mg/L at 25°C (Tewari et al. 1982), suggests that wet deposition may remove 2-butanone from the atmosphere. 2-Butanone has been identified in rain water (Grosjean and Wright 1983). The absence of significant amounts of particulate 2-butanone indicates that dry deposition to the earth's surface is not an important fate process. The short residence time expected for 2-butanone in the atmosphere, less than 1 day,suggests that it is not transported long distances from its original point of release.

Based on an experimental soil adsorption coefficient (K_{oc}) of 3.55 (Roy and Griffin 1985) 2-butanone is expected to display very high mobility in soil (Swann et al. 1983). 2-Butanone was found in groundwater samples shortly after a tanker spill (Halvorsen and Ohneck 1985) and in the groundwater underneath hazardous waste sites and public landfills (Francis et al. 1980; Sabel and Clark 1984; Sawhney and Kozloski 1984). The vapor pressure of 2-butanone, 90.6 mmHg at 25°C (Riddick et al. 1986), and the Henry's law constant, 5.77×10^{-5} atm m^3/mol at 25°C, suggest that volatilization from either dry or moist soil to the atmosphere will be an important environmental process.

If 2-butanone is released to water it is expected to rapidly volatilize to the atmosphere. Based on its Henry's law constant, an estimated volatilization half-life from a model river 1 m deep, flowing at 1 m/set with a wind velocity of 3 m/sec, is approximately 15 hours (Lyman et al. 1982).

2-Butanone is not expected to significantly adsorb to sediment and suspended organic matter. It is also not expected to bioconcentrate in fish and aquatic organisms (Lyman et al. 1982). These conclusions are based on an experimental K_{oc} of 3.55 (Roy and Griffin 1985), and a calculated bioconcentration factor of 0.98 obtained from its octanol/water partition coefficient, 0.29 (Hansh and Leo 1985), and an appropriate regression equation (Lyman et al. 1982).

5.3.2 Transformation and Degradation

5.3.2.1 Air

2-Butanone is expected to undergo atmospheric destruction by the gasphase reaction with photochemically produced hydroxyl radicals. Rate constants for this reaction ranging from 1.85×10^{-11} to 9.8×10^{-13} atm/molecule-sec in the temperature range of 22-32°C have appeared in the literature (Cox et al. 1980, 1981; Edney and Corse 1986; Edney et al. 1986; Darnall et al. 1976; Gusten et al. 1984; Wallington and Kurylo 1987; Wallington et al. 1988). Using a recommended rate constant of 1.85×10^{-11} atm/molecule-sec at 25°C and an average atmospheric hydroxyl radical concentration of 5×10^{5} molecule/cm^3 (Atkinson 1985), a half-life of 21 hours for this reaction can be calculated. However, experiments performed under simulated atmospheric conditions in the laboratory have shown that 2-butanone has a half-life of only 9.8 hours for photo-initiated processes (Dilling et al. 1976). The rate of its destruction increased in the presence of other anthropogenic compounds. The atmospheric destruction of 2-butanone as a result of direct irradiation is not expected to be significant under atmospheric conditions (Cox et al. 1980). Therefore, direct photolysis cannot account for the enhanced rate of atmospheric destruction observed in the laboratory. However, the data suggest that other mechanisms are responsible for the destruction of 2-butanone in the atmosphere, which are yet to be defined.

5.3.2.2 Water

2-Butanone is expected to be removed from environmental waters by microbial degradation under both aerobic and anaerobic conditions. Limited data specific to the chemical degradation of 2-butanone in water are available; however, it is not expected to occur to any significant extent.

Numerous investigations have concluded that 2-butanone undergoes biological degradation under aerobic conditions. At an initial concentration of 1 ppm, 2-butanone completely degraded in aerated water obtained from a deep Florida aquifer within 14 days after a 5-day lag period (Delfino and Miles 1985). Screening studies using a microbial seed from domestic waste treatment plants have indicated that 2-butanone has a 5-day biological oxygen demand (BOD_5) which is between 59% and 74% of the theoretical amount after a short lag period (Babeu and Vaishnav 1987; Bridie et al. 1979; Gaudy et al. 1963; Price et al. 1974; Urano and Kato 1986; Vaishnav et al. 1987; Young et al.

1968). A pure culture study indicated that propionate is produced as a result of the microbial oxidation of 2-butanone (Phillips and Perry 1974).

2-Butanone has been listed as a compound amenable to degradation by anaerobic biotechnology (Speece 1983). At an initial concentration of 500 ppm, 2-butanone was completely reduced to methane within 8 days in a fermentor using a domestic sludge inoculum that had been adapted to acetate (Chou et al. 1979).

An experimentally determined rate constant of 5.4×10^{8} L/mol-sec has been determined for the reaction of 2-butanone with hydroxyl radicals in water (Anbar and Neta 1967). This value corresponds to a half-life of 4 years for this reaction, given a hydroxy radical concentration of 1×10^{-17} M (Mill et al. 1980). Hydrolysis of ketones is generally not believed to be an environmentally important process (Lyman et al. 1982; Mill 1982). A rate constant of 0 L/mol-year was listed for the hydrolysis of 2-butanone under neutral, acidic, and basic conditions at 25°C (Kollig et al. 1987), indicating that this process does not occur in the environment. By analogy to the gasphase photolysis of 2-butanone (Cox et al. 1980), direct photochemical breakdown of 2-butanone in water is not expected. Therefore, the chemical degradation of 2-butanone in environmental waters is not expected to occur to any significant extent.

The chemical alteration of 2-butanone in rain water has been postulated. In acid rain, hydroxy sulfonates may be formed by the reaction with bisulfite, and ammonia adducts may be formed in ammoniated rain (Grosjean and Wright 1983). The concentration of these reactive species is likely to be much higher in rain water than in surface water; therefore, a more rapid rate of reaction would be expected in rain.

5.3.2.3 Soil

No specific data concerning the fate of 2-butanone in soil were available. By analogy to the experimental results on the microbial degradation of 2-butanone in water, this compound may degrade in soil under aerobic and anaerobic conditions given suitable time for adaptation of the microbial population. Again by using an analogy to the fate of 2-butanone in aqueous systems, it is not expected to hydrolyze, photolyze on the surface, or undergo chemical degradation.

5.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

5.4.1 Air

2-Butanone has been detected in a limited number of sites in rural, urban, and indoor locations. It was detected in 17 samples taken in Tucson, Arizona, in 1982 at an average concentration of 2.8 ppb. In the mountains of Arizona, the concentration was 0.50 ppb (Snider and Dawson 1985). The range

of 2-butanone measured in Los Angeles air in 1980 was.O-14 ppb in 70 samples (Grosjean 1982). 2-Butanone was found in one-third of samples taken downwind of a solvent recycling facility in Maryland in 1970, at a maximum concentration of 94 ppm (Smoyer et al. 1971). Although it has been detected in the exhaust of gasoline engines, it was not found in the air of a highway mountain tunnel (Hampton et al. 1982).

2-Butanone was detected in the air of the Kin-But chemical waste site, located in New Jersey, at concentrations ranging from trace to 1.5 pg/m3 (0.51 ppb), and 0.5 to 33 $\mu g/m^3$ (0.17-11.3 ppb) in samples surrounding the site (Pellizzari 1982). It was qualitatively detected in the air at four of four hazardous waste sites and one landfill in New Jersey (LaRegina et al. 1986).

In a survey of 36 homes taken in Chicago, Illinois, 2-butanone was detected in the indoor air at 3 residences (Jarke et al. 1981). It was also found in three outdoor samples in this survey. It is not clear, however, if the positive indoor and outdoor samples were collected at the same location. In a compilation and analysis of ambient monitoring data collected from 1970 to 1987, the daily concentration of 2-butanone was 0 ppb in urban, suburban, and rural areas (Shah and Heyerdahl 1988). 2-Butanone has been qualitatively detected in the indoor air of homes in Chicago (Jarke et al. 1981) and in Canadian residential and office buildings (Tsuchiya 1987).

The sporadic ambient air monitoring data available for 2-butanone suggest that the average background concentration of this compound may be very low. However, the available data also suggest that there are dramatic, temporal, and diurnal variations in its concentration.

5.4.2 Water

Numerous studies have qualitatively detected 2-butanone in drinking water supplies (Kool et al. 1982). It has been found in drinking water from the District of Columbia; Cincinnati, Ohio; Miami, Florida; Ottumwa, Iowa; Philadelphia, Pennsylvania; Seattle, Washington; Tuscaloosa, Alabama; and New Orleans, Louisiana (Bertsch et al. 1975; Coleman et al. 1976; EPA 1974, 1975; Kopfler et al. 1977; Scheiman et al. 1974). It was detected in Des Moines, Iowa, drinking water samples at an estimated concentration of 1.6 ppb (Ogawa and Fritz 1985). 2-Butanone was detected in tap water 8 months after the installation of new polyvinyl chloride (PVC) pipes at a concentration ranging from 0.4 to 4.5 ppm (Wang and Bricker 1979). It resulted from the glue used to cement the water pipes together. The concentration of 2-butanone in the water increased with the amount of time the water sat in the pipes.

2-Butanone has been qualitatively detected in rain water and the clouds of Henninger, California, at 0.04 ppb, and in the mist of Long Beach, California (Grosjean and Wright 1983). Trace amounts have also been found in the ice in Fairbanks, Alaska (Grosjean and Wright 1983).

2-Butanone was listed as being detected in less than 5% of U.S. groundwater supplies (Dyksen and Hess 1982). At U.S. hazardous waste sites, 2-Butanone was listed as being frequently detected in the groundwater (Garman et al. 1987). This statement should be interpreted with caution, as "frequently" was defined as greater than 0.1%, of the samples. 2-Butanone was detected in 180 groundwater samples from 357 hazardous waste sites monitored by the CLP at a geometric mean concentration of 302 ppb in the positive samples (CLPSD 1988). Examples of.the presence of 2-butanone at hazardous waste sites and landfills can be found in Table 5-2. It was detected in groundwater samples underneath a tanker truck spill at concentrations up to 2,200 ppm (Halvorsen and Ohneck 1985). Interpretation of the concentration of 2-butanone found in groundwater samples should be made carefully, and should take into account the experimental methods used in the determinations; results may be skewed due to the presence of 2-butanone in the adhesives used to cement PVC well pipes together (Sosebee et al. 1983).

2-Butanone has been detected in the effluent of various industrial processes. It was found in six of seven waste water samples from energyrelated processes at a concentration up to 645 ppb (Pellizzari et al. 1979). 2-Butanone was detected in the waste water of a specialty chemical manufacturing plant at a concentration of 8-20 ppm, but not in the receiving river water or its sediment (Jungclaus et al. 1978). It was also detected in the waste water from shale oil processing at a concentration of 0.4-18 ppm (Hawthorne and Sievers 1984).In 1982, 2-butanone was detected at concentrations of 83 ppb or less in the waste water entering Cincinnati treatment plants (Dunovant et al. 1986).

2-Butanone has been detected in 77 of 357 surface water samples at U.S. hazardous waste sites at a geometric mean concentration of 11 ppb for the positive samples (CLPSD 1988). It was qualitatively detected in the Black Warrier River, located in Tuscaloosa, Alabama (Bertsch et al. 1975), and in sea water from the straits of Florida at O-22 ppb in 1968 (Corwin 1969).

5.4.3 Soil

Limited data are available on the detection of 2-butanone in soil samples. It has been found in 309 of 357 hazardous waste sites monitored by the CLP at a geometric mean concentration of 87 ppb for the positive samples (CLPSD 1988).

5.4.4 Other Environmental Media

2-Butanone has been detected as a natural component of numerous types of foods. It has been qualitatively identified as a volatile constituent in raw chicken breast muscle, milk, roasted filberts (nuts), Beaufort (Gruyere) and cheddar cheese, bread dough, and intact tree-ripened nectarines (Dumont and Adda 1978; Gordon and Morgan 1979; Grey and Shrimpton 1967; Keen et al. 1974;

TABLE 5-2. Detection of 2-Butanone in the Groundwater of Hazardous Waste Sites and Landfills

Type/Location	Sampling Dates	No. of Samples	No. of Positive	Concentration	Reference
Waste sites, groundwater					
Connecticut	1982-1983	5	1	4,800 ppm	Sawhney and Kozloski 1984
Low-level radioactive					
Waste sites, surface water					
Valley of the Drums, KY	No data	No data	No data	≤690 ppb	Stonebraker and Smith 1980
Landfills, groundwater					
Municipal solid waste	No data	13	7	6.8-6,200 ppb	Sabel and Clark 1984
Landfills, leachate					
Municipal solid waste	No data	6	6	110-27,000 ppb	Sabel and Clark 1984

Kinlin et al. 1972; Sosulski and Mahmoud, 1979; Takeoka 'et al. 1988). The mean concentration of 2-butanone in dried beans, split peas, and lentils was 148, 110, and 50 ppm, respectively (Lovegren et al. 1979). 2-Butanone has been detected in southern peas at a median concentration of 120 ppb (Fisher et al. 1979), and it has been qualitatively detected in winged beans and soybeans (Del Rosario et al. 1984). It has also been detected in cigarette smoke (Higgins et al. 1983; Osborne et al. 1956).

5.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

Available monitoring data suggest that the general population is exposed to 2-butanone. In the early stages of the Total Exposure Assessment Methodology (TEAM) study, 2-butanone was qualitatively detected in 3 of 8 personal air samples, 5 of 12 breath samples, and 1 of 1 drinking water sample obtained from 12 volunteers living in urban areas of New Jersey or North Carolina (Wallace et al. 1984). 2-Butanone has also been detected in the expired air of 206 of 387 samples (53.2%) taken from 54 adult, nonsmoking, urban dwelling subjects, at an average concentration of 3.6 ng/L (Krotoszynski et al. 1979). It was detected in the expired air of six of eight male volunteers, three of whom were smokers (Conkle et al. 1975). 2-Butanone was found in 5 of 12 samples of human mothers' milk from subjects in 4 different U.S. urban areas (Pellizzari et al. 1982). It has been qualitatively detected in the indoor air of homes in Chicago (Jarke et al. 1981) and in Canadian residential and office buildings (Tsuchiya 1987).

Exposure to 2-butanone by the general population may occur by ingestion of contaminated drinking water. This compound has been identified in U.S. drinking water supplies (Bertsch et al. 1975; Coleman et al. 1976; EPA 1974, 1975; Kopfler et al. 1977; Ogawa and Fritz 1985; Scheiman et al. 1974). Inhalation is also a likely route of exposure to 2-butanone, especially during the household use of commercial coatings that use 2-butanone as a solvent. Exposure by dermal contact may also occur during the use of such coatings.

2-Butanone is a naturally occurring constituent in a variety of common foods (Del Rosario et al. 1984; Dumont and Adda 1978; Gordon and Morgan 1979; Grey and Shrimpton 1967; Keen et al. 1974; Kinlin et al. 1972; Lovegren et al. 1979; Takeoka et al. 1988). Ingestion of these foods will result in exposure to 2-butanone. Exposure to 2-butanone may also occur while smoking (Higgins et al. 1983; Osborne et al. 1956). Students taking undergraduate general chemistry laboratory courses may be also exposed to 2-butanone (Kolb 1988).

According to the National Occupational Exposure Survey (NOES) conducted by NIOSH between 1980 and 1983, 1,221,587 workers, of which 201,308 were women, were potentially exposed to 2-butanone during that time period (NIOSH 1989). Of these workers, 84% (80% for the women) were exposed during the use of trade name products containing 2-butanone. Occupational exposure is expected to occur by inhalation and dermal contact.

A study of three companies involved in spray painting and spray gluing operations reported that, for 89 workers exposed to 2-butanone, the mean air concentration was 0.3 ppm (Whitehead et al. 1984). 2-Butanone was detected in the air of Cincinnati waste water treatment plants in 1982; 3 of 17 samples were positive at concentrations of 5.7 ppb or less (Dunovant et al. 1986). It has also been detected in the air above shale oil waste waters (Hawthorne and Sievers 1984). The breathing zone air for workers at an organic solvent recycling plant averaged 11 ppm during drum decantation operations, and 10 ppm during all other work activities (Kupferschmid and Perkins 1986). The ambient concentration was not greater than exposure limits of 200 ppm in any of these examples (NIOSH 1984). The concentrations of 2-butanone in air samples obtained from the Skylab, 1973-74, ranged from 2.4 to 1,505 ppb (Liebich et al. 1975). Personal exposure to 2-butanone at a waste solvent incineration facility ranged from <O.Ol to 1.2 ppm (Decker et al. 1983).

5.6 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

For the general population, high levels of exposure to 2-butanone may occur for those living near commercial settings where this compound is used. For example, the downwind 2-butanone concentration near a solvent recycling facility was measured at concentrations up to 94 ppm (Smoyer et al. 1971). High levels of exposure may also occur during the use of commercial coatings containing 2-butanone, especially when working in enclosed, unventilated spaces. Members of the general population living near hazardous waste sites and drawing their drinking water from groundwater sources may be exposed to high levels of 2-butanone through ingestion of contaminated water, although no information on the size of the population can be provided.

High levels of occupational exposure to 2-butanone may occur by inhalation and dermal contact during the loading and unloading of large quantities of this material during shipment. The application of commercial coatings containing 2-butanone without adequate protection may also lead to high levels of exposure, primarily by inhalation.

5.7 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA as amended directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of 2-butanone is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of 2-butanone.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that, if met, would reduce or eliminate

the uncertainties of human health assessment. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

5.7.1 Data Needs

Physical and Chemical Properties. The physical and chemical properties of 2-butanone are well documented. The environmental fate of 2-butanone can be predicted from these properties and compared to experimental results once they are obtained in areas where deficiencies exist.

Production, Import/Export, Use, and Disposal. The significant amounts of 2-butanone produced in the United States, combined with its prevalence in commercial and household products, suggest that large numbers of citizens are potentially exposed to,anthropogenic sources of this $ompound. The production, use, and international trading of 2-butanone is well described in the available literature (Chemical Marketing Reporter 1987; Neir and Strehlke 1985; Papa and Sherman 1981; USITC 1987, 1988, 1989). Methods for the disposal of 2-butanone are established (HSDB 1989; OHM/TADS 1989), but the amounts processed by each method cannot be ascertained. Therefore, disposal of 2-butanone cannot be compared to the regulations controlling this practice. Knowing the amount of 2-butanone released to the environment and its disposal pattern will aid in determining routes and levels of exposure to the general population by indicating which media should be monitored carefully.

According to the Emergency Planning and Community Right-to-Know Act of 1986, 42 U.S.C. Section 11023, industries are required to submit chemical release and off-site transfer information to EPA. The Toxic Release Inventory (TRI), which contains this information for 1987, became available in May of 1989. This database will be updated yearly and should provide a list of industrial production facilities and emissions.

Environmental Fate. There is sufficient predictive information to indicate that 2-butanone is not likely to partition from water (Hansch and Leo 1985; Lyman et al. 1982; Roy and Griffen 1985); yet, there are few field studies to verify these predictions. Similarly, 2-butanone's transport, transformation, and degradation in the environment can be predicted (Atkinson 1985; Babeu and Vaishnav 1987; Cox et al. 1980; Delfino and Miles 1985), but not as yet experimentally substantiated in all areas. Experimental studies in this area would allow the determination of 2-butanone's lifetime in the environment and aid in determining levels and routes of human exposure.

Bioavailability from Environmental Media. Numerous toxicokinetic and toxicity studies in humans and animals have demonstrated the bioavailability of 2-butanone from air, ingestion of food and water, and dermal contact. Absorption of 2-butanone after inhalation is well established, and it appears to be adsorbed after ingestion. These mechanisms are consistent with what one would expect, based on 2-butanone's physical and chemical properties (Lyma

et al. 1982). Given the potential for exposure to 2-butanone because of its prevalence in commercial products available to the public (Neier and Strehlke 1985) and its ability to enter the immediate areas of the environment (TRI 1989), further research on the bioavailability of this compound will allow the quantification of human exposure and risk.

Food Chain Bioaccumulation. 2-Butanone is not believed to appreciably bioconcentrate in fish and aquatic organisms (Hansch and Leo 1985; Lyman et al. 1982). It is also not expected to biomagnify in the food chain. Quantitative data supporting these conclusions are not available in the literature. Additional information on bioconcentration and biomagnification would be useful in confirming the predicted behavior of this compound.

Exposure Levels in Environmental Media. Data are available regarding the level of 2-butanone in endronmental media (Grosjean and Wright 1983; Shah and Heyerdahl 1988) and foods (Dumont and Adda 1978; Grey and Shrimpton 1967; Kinlin et al. 1972; Lovegren et al. 1979; Takeoka et al. 1988); however, the data available are often qualitative and only generalized trends regarding the occurrence of this compound can be derived. Its presence in environmental media near hazardous waste sites is not well documented (CLPSD 1988). Quantitative determination of the levels of 2-butanone in environmental media and foods will allow the estimation of levels on human intake of this compound from each media.

Exposure Levels in Humans. 2-Butanone has been found in the human blood samples of urban dwellers, but the observed levels have not been correlated with personal activities. Studies on the level of 2-butanone in human tissues near hazardous waste sites are not complete. A correlation of the levels of 2-butanone in humans with their personal activities or the areas where they live will allow an assessment of potential exposure to the general population. Similarly, correlations of occupational exposure by profession will allow a determination of human exposure levels.

Exposure Registries. No exposure registries for 2-butanone were located. This compound is not currently one of the compounds for which a subregistry has been established in the National Exposure Registry. The compound will be considered in the future when chemical selection is made for subregistries to be established. The information that is amassed in the National Exposure Registry facilitates the epidemiological research needed to assess adverse health outcomes that may be related to the exposure to the compound.

5.7.2 On-going Studies

On-going studies on water purification techniques and transformation of 2-butanone in the environment have been identified (EPA 1989b), although no specific information was provided.

As part of the Third National Health and Nutrition Evaluation Survey (NHANES III), the Environmental Health Laboratory Sciences Division of the Center for Environmental Health and Injury Control, Centers for Disease Control, will be analyzing human blood samples for 2-butanone and other volatile organic compounds. These data will give an indication of the frequency of occurrence and background levels of these compounds in the general population.

6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting and/or measuring and monitoring 2-butanone in environmental media and in biological samples. The intent is not to provide an exhaustive list of analytical methods that could be used to detect and quantify 2-butanone. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used to detect 2-butanone in environmental samples are the methods approved by federal agencies such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that refine previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

6.1 BIOLOGICAL MATERIALS

Numerous procedures that detect-2-butanone in biological materials have been reported in the literature. A summary of.these methods is found in Table 6-l. In general, each technique appears to be capable of determining 2-butanone in any type of biological matrix given suitable modification of the sample collection/preparation step.

Since 2-butanone is a volatile compound, analysis by headspace sampling can be accomplished for both liquid matrices, such as blood and urine (Deveaus and Huvenne 1987; Perbellini et al. 1984), and solid matrices, such as soft tissue (Perbellini et al. 1984). For solid samples such as soft tissue, better results are obtained if the sample is first homogenized at low temperatures before analysis. The technique involves gently heating the sample in a closed system, followed by withdrawing a portion of the air above the sample (the headspace) by syringe. Separation of 2-butanone from other compounds that may be present is then accomplished by injecting the contents of the syringe directly into a gas chromatograph (GC). As indicated in Table 6-2, successful quantification of 2-butanone has been accomplished using a variety of detection systems, including a flame ionization detector, mass spectrometer, or Fourier transform infrared spectrometer.

Analysis of 2-butanone in liquid samples can also be accomplished by purge and trap methodology (Pellizzari et al. 1982). In this technique, an inert gas is bubbled through the sample, liberating 2-butanone. The contaminant is then trapped on an adsorbent cartridge, followed by thermal desorption directly into a gas chromatograph (GC). This technique has been successfully used for analysis of human milk samples (Pellizzari et al. 1982)

Extractive techniques can also be used for the analysis of 2-butanone in liquid samples (Kezic and Monster 1988; Van Doorn et al. 1989). The sample is shaken with an immiscible organic solvent into which 2-butanone partitions.

TABLE 6-1. Analytical Methods for Determining 2-Butanone in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit (ppm)	Percent recovery	Reference
Urine	Derivatization with o-nitrophenol-hydrazine, liquid-liquid extraction by HPLC	HPLC/UV	0.10	No data	Van Doorn et al. 1989
Urine	Acidification, extraction with CH_2Cl_2 by HPLC, concentration	GC/FID	0.01	85%-91%	Kezic and Monster 1988
Urine	Headspace analysis	GC/MS	No data	No data	Perbellini et al. 1984
Blood	Heat sample to 80°C in sealed tube, obtain headspace sample	GC/FTIR	6	No data	Deveaux and Huvenne 1987
Blood	Derivatization with o-nitrophenyl-hydrazine, liquid-liquid extraction by HPLC	HPLC/UV	0.10	No data	Van Doorn et al. 1989
Blood	Headspace analysis	GC/MS	No data	No data	Perbellini et al. 1984
Milk	Purge at elevated temperatures, trap on Tenax cartridges, thermal desorption	GC/MS	No data	No data	Pellizzari et al. 1982
Soft tissue	Homogenize sample at 4°C, heat to 80°C in sealed tube, obtain headspace sample	GC/FTIR	6	No data	Deveaux and Huvenne 1987
Soft tissue	Headspace analysis	GC/MS	No data	No data	Perbellini et al. 1984
Expired air	Collect breath in Tedlar bag pump air through Tenax cartridges, thermal desorption	GC/MS	No data	No data	Wallace et al. 1984

FID = flame ionization detector
FTIR = Fourier transform infrared spectrometry
GC = gas chromatography
HPLC = high performance liquid chromatography
MS = mass spectrometry
UV = ultraviolet spectroscopy

TABLE 6-2. Analytical Methods for Determining 2-Butanone in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Pump air through sorbent tube, desorb with CS_2 - NIOSH 2500	GC/FID	4 μg	92%	NIOSH 1984
Air	Pump air through cryogenic trap, attach trap to GC, thermal desorption	GC/FID	8 ppb	No data	Jonsson et al. 1985
Water	Purge and trap	GC/FID GC/MS	10 ppb	No data	EPA 1988
Drinking water	Purge and trap on a Tenax cartridge	GC/FID	No data	No data	Wallace et al. 1984
Soil	Add water, heat to 40°, purge and trap, thermal desorbtion	GC/FID GC/MS	10 ppb	No data	EPA 1988
Sediment	Add water, heat to 40°, purge and trap, thermal desorbtion	GC/FID GC/MS	10 ppb	No data	EPA 1988

FID = flame ionization detector
GC = gas chromatography
MS = mass spectrometry

After concentrating the extract, the sample can be analyzed by different methods. 2-Butanone can first be derivitized to increase its extraction efficiency, or to make it more visible to the detection system.

Numerous types of detection and quantitation methods have been used in the analysis of 2-butanone in biological samples. Gas chromatography has been used to separate 2-butanone from other contaminants that may be present. Direct quantitation can be made by using a flame ionization detector (FID), tandem gas chromatography-mass spectrometry (GC-MS), or tandem GC-fourier transform infrared spectrometry (GC-FTIR). The latter two techniques also allow direct identification of the contaminants. Analysis of derivitized 2-butanone can be accomplished using high-performance liquid chromatography (HPLC) equipped with an ultraviolet (WV) detector. These detection systems are capable of measuring 2-butanone in the sub-ppm range; the limits on the GC-FTIR system are slightly greater than 1 ppm.

Given the numerous techniques available for the determination of 2-butanone in biological samples, and the lack of any standardized method for each individual matrix, the choice of an analysis technique appears to be a function of the instrumentation and personal biases of the laboratory performing the analysis. With the exception of the derivitization method, all of the techniques described above are also capable of determining the metabolites of 2-butanone (3-hydroxy-2-butanone, 2-butanol, and 2,3-dihydroxybutane) in biological samples.

6.2 ENVIRONMENTAL SAMPLES

A short description of standardized methods that can be used for the analysis of 2-butanone in environmental samples is presented in Table 6-2. It should be noted that an extensive list of methods for the analysis of 2-butanone in environmental samples can be compiled from the literature. In all of these methods, however, there is a consensus that, after the sample preparation stage, mixture separation and quantitative analysis are best determined by the use of a gas chromatograph coupled with any of an assortment of detectors (Bertsch et al. 1975; Coleman et al. 1976; Corwin 1969; Dunovant et al. 1986; Hawthorne and Sievers 1984; Isidorov et al. 1985; Jonsson et al. 1985; Jungclaus et al. 1978; LaRegina et al. 1986; Pellizzari 1982; Sawhney and Kozloski 1984; Smoyer et al. 1971; Snider and Dawson 1985; Wallace et al. 1984; Wang and Bricker 1979).

The analysis of 2 butanone in air can be accomplished by NIOSH method 2500 (NIOSH 1984). The sample is obtained in the field by the use of a pumping system to pass a measurable quantity of air (approximately l-12 L) through a tube loaded with a solid sorbent, Ambersorb XE-347. Extraction of the tube with the solvent carbon disulfide liberates the 2-butanone, and quantitation is achieved by GC using a flame ionization detector.

6. ANALYTICAL METHODS

The analysis of 2-butanone in soil, sediment, and water samples at hazardous waste sites is described in the Contract Laboratory Program manual (EPA 1988). For the soil and sediment samples, the procedure begins with the addition of a small portion of water to the sample. At this point, all three matrices are subjected to a purge and trap cycle. An inert gas is bubbled through the sample, volatilizing 2-butanone. The gas stream is then passed through an adsorbent tube that recollects the 2-butanone. The sorbent tube is attached to a gas chromatograph and heated to flush the sample onto a GC column. Quantification can be accomplished using either a flame ionization detector or a mass spectrometer, depending on the total concentration of organics in the sample. Required quantitation limits for this program are 10 ppm in all three matrices. No other standardized methods for the determination of 2-butanone in environmental samples were located.

6.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA as amended directs the Administrator of ATSDR in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of 2-butanone is available. Uhere adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of 2-butanone.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that, if met, would reduce or eliminate the uncertainties of human health assessment. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.3.1 Data Needs

Methods for Determining Biomarkers of Exposure and Effect. Numerous methods for the determination of 2-butanone in biological materials have been described in the literature (Deveaux and Huvenne 1987; Kezic and Monster 1988; Pellizzari et al. 1982; Perbellini et al. 1984; Van Doorn et al. 1989). These methods are also appropriate for determining the metabolites of 2-butanone, 3-hydroxy-2-butanone, 2-butanol, and 2,3-dihydroxybutane, which also serve as biomarkers of exposure. These methods display the requisite sensitivity to measure background levels in the population and levels which may indicate a concern for biological effects. Standardized methods for these procedures should be established as no standardized methods could be located. Any methodology established for determining biomarkers of exposure must take into account the short biological half-life of 2-butanone, which may render these methods inferior to direct measurements of exposure.

There are no known biomarkers of effect which are specific to 2-butanone.

Methods for Determining Parent Compounds and Degradation Products in Snvironmental Media. Numerous methods capable of detecting low levels of 2-butanone in environmental media have been described in the literature (Bertsch et al. 1975; Dunovant 1985; Hawthorne and Sievers 1984; LaRegina et al. 1986; Pellizzari 1982; Wallace et al. 1984). Appropriate standardized methods are available for the determination of 2-butanone in air (NIOSH 1984), water, soil, and sediments (EPA 1988) at levels that may constitute a concern for human health. The methods for determining 2-butanone in air, the medium that represents the most concern for exposure, are highly sensitive, accurate, and reproducible. The exact levels of detection for these methods in all media, however, are not described. There are numerous reliable methods available in the literature that have been used to determine levels of 2-butanone in environmental media and foods at levels that may cause health effects to occur, but they have not been standardized.

6.3.2 On-going Studies

The Environmental Health Laboratory Sciences Division of the Center for Environmental Health and Injury Control, Centers for Disease Control, is developing methods for the analysis of 2-butanone and other volatile organic compounds in blood, These methods use purge and trap methodology and magnetic sector mass spectrometry which gives detection limits in the low parts per trillion range.

On-going studies involving the development of analytical methods have been identified (EPA 1989b), although no specific information was provided.

7. REGULATIONS AND ADVISORIES

International (World Health Organization) guidelines for 2-butanone were not located. National and state regulations and guidelines pertinent to human exposure to 2-butanone are summarized in Table 7-1.

7. REGULATIONS AND ADVISORIES

TABLE 7-1. Regulations and Guidelines Applicable to 2-Butanone

Agency	Description	Information	References
NATIONAL			
Regulations:			
a. Air:			
OSHA	PEL	200 ppm	OSHA 1989 (29 CFR 1910.0000)
	STEL	300 ppm	
b. Nonspecific media:			
EPA OERR	Reportable quantity	5,000 pounds	EPA 1985a (40 CFR 117 and 302)
Guidelines:			
a. Air:			
ACGIH	TLV TWA	200 ppm	ACGIH 1989
	STEL	300 ppm	
NIOSH	Occupational standard	200 ppm	NIOSH 1978
b: Other:			
EPA	RfD (oral)	$5x10^{-2}$ mg/kg/day	EPA 1989c
STATE			
Regulations and Guidelines:			
a. Air:	Acceptable ambient air concentrations		
Connecticut		11,800 $\mu g/m^3$ (8-hr avg)	NATICH 1988
Kentucky		295 mg/m^3 (8-hr avg)	State of Kentucky 1986
Florida		5.9 mg/m^3 (8-hr avg)	NATICH 1988
Massachusetts		160 $\mu g/m^3$ (24-hr avg)	NATICH 1988
North Carolina		88.5 mg/m^3 (15-min avg)	NATICH 1988
		3.7 mg/m^3 (24-hr avg)	NATICH 1988
North Dakota		5.9 mg/m^3 (8-hr avg)	NATICH 1988
		8.85 mg/m^3 (24-hr avg)	NATICH 1988
Nevada		14.048 mg/m^3 (8-hr avg)	NATICH 1988
New York		1,967 $\mu g/m^3$ (annual)	NATICH 1988
South Carolina		14,750 $\mu g/m^3$ (24-hr avg)	NATICH 1988
South Dakota		11,800 $\mu g/m^3$ (8-hr avg)	NATICH 1988
Virginia		9,800 $\mu g/m^3$ (24-hr avg)	NATICH 1988
b. Water:	Drinking water quality guidelines		
Arizona		170 $\mu g/L$	FSTRAC 1988
Connecticut		1,000 $\mu g/L$	FSTRAC 1988
Massachusetts		350 $\mu g/L$	MAORS 1989
Maine		750 $\mu g/L$	FSTRAC 1988
Minnesota		172 $\mu g/L$	FSTRAC 1988
	Ground water quality standards		
Vermont	Enforcement standard	170 $\mu g/L$	VDEC 1990
	Preventive action limit	85 $\mu g/L$	VDEC 1990

ACGIH = American Conference of Governmental Industrial Hygienists; EPA = Environmental Protection Agency; NIOSH = National Institute for Occupational Safety and Health; OERR = Office of Emergency and Remedial Response; OSHA = Occupational Safety and Health Administration; PEL = Permissible Exposure Limit; RfD = Reference dose; STEL = Short Term Exposure Limit; TLV = Threshold Limit Value; TWA = Time-Weighted Average

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9. GLOSSARY

Acute Exposure -- Exposure to a chemical for a duration of 14 days or less, as specified in the Toxicological Profiles.

Adsorption Coefficient (K_{oc}) -- The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

Adsorption Ratio (Kd) -- The amount of a chemical adsorbed by a sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

Bioconcentration Factor (BCF) -- The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

Cancer Effect Level (CEL) -- The lowest dose of chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or tumors) between the exposed population and its appropriate control.

Carcinogen -- A chemical capable of inducing cancer.

Ceiling Value -- A concentration of a substance that should not be exceeded, even instantaneously.

Chronic Exposure -- Exposure to a chemical for 365 days or more, as specified in the Toxicological Profiles.

Developmental Toxicity -- The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Embryotoxicity and Fetotoxicity -- Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the insult occurred. The terms, as used here, include malformations and variations, altered growth, and in utero death.

EPA Health Advisory -- An estimate of acceptable drinking water levels for a chemical substance based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

9. GLOSSARY

Immediately Dangerous to Life or Health (IDLH) -- The maximum environmental concentration of a contaminant from which one could escape within 30 min without any escape-impairing symptoms or irreversible health effects.

Intermediate Exposure -- Exposure to a chemical for a duration of 15-364 days as specified in the Toxicological Profiles.

Immunologic Toxicity -- The occurrence of adverse effects on the immune system that may result from exposure to environmental agents such as chemicals.

In vitro -- Isolated from the living organism and artificially maintained, as in a test tube.

In vivo -- Occurring within the living organism.

Lethal Concentration $_{(Lo)}$($LC_{(Lo)}$ -- The lowest concentration of a chemical in air which has been reported to have caused death in humans or animals.

Lethal Concentration$_{(50)}$ (LC_{50}) -- A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose$_{(Lo)}$ (LD_{Lo}) -- The lowest dose of a chemical introduced by a route other than inhalation that is expected to have caused death in humans or animals.

Lethal Dose$_{(50)}$ (LD_{50}) -- The dose of a chemical which has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time$_{(50)}$ ($LT_{(50)}$) -- A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL) -- The lowest dose of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Malformations -- Permanent structural changes that may adversely affect survival, development, or function.

Minimal Risk Level -- An estimate of daily human exposure to a chemical that is likely to be without an appreciable risk of deleterious effects (noncancerous) over a specified duration of exposure.

Mutagen -- A substance that causes mutations. A mutation is a change in the genetic material in a body cell. Mutations can lead to birth defects, miscarriages, or cancer.

9. GLOSSARY

Neurotoxicity -- The occurrence of adverse effects on the nervous system following exposure to chemical.

No-Observed-Adverse-Effect Level (NOAEL) -- The dose of chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Effects may be produced at this dose, but they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{ow}) -- The equilibrium ratio of the concentrations of a chemical in n-octanol and water, in dilute solution.

Permissible Exposure Limit (PEL) -- An allowable exposure level in workplace air averaged over an 8-hour shift.

q_1*-- The upper-bound estimate of the low-dose slope of the dose-response curve as determined by the multistage procedure. The q_l* can be used to calculate an estimate of carcinogenic potency, the incremental excess cancer risk per unit of exposure (usually *ug*/L for water, mg/kg/day for food, and $\mu g/m^3$ for air).

Reference Dose (RfD) -- An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure of the human population to a potential hazard that is likely to be without risk of deleterious effects during a- lifetime. The RfD is operationally derived from the NOAEL (from animal and human studies) by a consistent application of uncertainty factors that reflect various types of data used to estimate RfDs and an additional modifying factor, which is based nn a professional judgment of the entire database on the chemical. The RfD are not applicable to nonthreshold effects such as cancer.

Reportable Quantity (RQ) -- ,The quantity of a hazardous substance that is considered reportable under CERCLA. Reportable quantities are (1) 1 lb or greater or (2) for selected substances, an amount established by regulation either under CERCLA or under Sect. 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

Reproductive Toxicity -- The occurrence of adverse effects on the reproductive system that may result from exposure to a chemical. The toxicity may bedire.cted to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

9. GLOSSARY

Short-Term Exposure Limit (STEL) -- The maximum concentration to which workers can be exposed for up to 15 min continually. No more than four excursions are allowed per day, and there must be at least 60 min between exposure periods. The daily TLV-TWA may not be exceeded.

Target Organ Toxicity -- This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen -- A chemical that causes structural defects that affect the development of an organism:

Threshold Limit Value (TLV) -- A concentration of a substance to which most workers can be exposed without adverse effect. The TLV may be expressed as a TWA, as a STEL, or as a CL.

Time-Weighted Average (TWA) -- An allowable exposure concentration averaged over a normal 8-hour workday or 40-hour workweek.

Toxic Dose (TD_{50}) -- A calculated dose of a chemical, introduced by a route other than inhalation, which is expected to cause a specific toxic effect in 50% of a defined experimental animal population.

Uncertainty Factor (UF) -- A factor used in operationally deriving the RfD from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using LOAEL data rather than NOAEL data. Usually each of these factors is set equal to 10.

APPENDIX A

USER'S GUIDE

Chapter 1

Public Health Statement

This chapter of the profile is a health effects summary written in nontechnical language. Its intended audience is the general public especially people living in the vicinity of a hazardous waste site or substance release. If the Public Health Statement were removed from the rest of the document, it would still communicate to the lay public essential information about the substance.

The major headings in the Public Health Statement are useful to find specific topics of concern. The topics are written in a question and answer format. The answer to each question includes a sentence that will direct the reader to chapters in the profile that will provide more information on the given topic.

Chapter 2

Tables and Figures. for Levels of Significant Exposure (LSE)

Tables (2-1, 2-2, and 2-3) and figures (2-l and 2-2) are used to summarize health effects by duration of exposure and endpoint and to illustrate graphically levels of exposure associated with those effects. All entries in these tables and figures represent studies that 'provide reliable, quantitative estimates of No-Observed-Adverse-Effect Levels (NOAELs), Lowest-Observed- Adverse-Effect Levels (LOAELS) for Less Serious and Serious health effects, or Cancer Effect Levels (CELs). In addition, these tables and figures illustrate differences in response by species, Minimai Risk Levels (MRLs) to humans for noncancer end points, and EPA's estimated range associated with an upper-bound individual lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. The LSE tables and figures can be used for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text.

The legends presented below demonstrate the application'of these tables and figures. A representative example of LSE Table 2-1 and Figure 2-l are shown. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

LEGEND

See LSE Table 2-l

1) Route of Exposure One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. When sufficient data exist,

three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure, i.e., inhalation, oral, and dermal (LSE Table 2-1, 2-2, and 2-3, respectively). LSE figures are limited to the inhalation LSE Figure 2-1) and oral (LSE Figure 2-2) routes.

(2) Exposure Duration Three exposure periods: acute (14 days or less); intermediate (15 to 364 days); and chronic (365 days or more) are presented within each route of exposure. In this example, an inhalation study of intermediate duration exposure is reported.

(3) Health Effect The major categories of health effects included in LSE tables and figures are death, systemic, immunological, neurological, developmental, reproductive, and cancer. NOAELs and LOAELs can be reported in the tables and figures for all effects but cancer.- Systemic effects are further defined in the "System" column of the LSE table.

(4) Key to Figure Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 18 has been used to define a NOAEL and a Less Serious LOAEL (also see the two “18r” data points in Figure 2-l).

(5) Species The test species, whether animal or human, are identified in this column.

(6) Exposure Freauency/Duration The duration of the study and the weekly and daily exposure regimen are provided in this column. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 18), rats were exp'osed to [substance x] via inhalation for 13 weeks, 5 days per week, for 6 hours per day.

(7) System This column further defines the systemic effects. These systems include: respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, and dermal/ocular. "Other" refers to any systemic effect (e.g., a decrease in body weight) not covered in these systems. In the example of key number 18, one systemic effect (respiratory) was investigated in this study.

(8) NOAEL A No-Observed-Adverse-Effect Level (NOAEL) is the highest exposure level at which no harmful effects were seen in the organ system studied. Key number 18 reports a NOAEL of 3 ppm for the respiratory system which was used to derive an intermediate exposure, inhalation MRL of 0.005 ppm (see footnote Wc").

(9) LOAEL A Lowest-Observed-Adverse-Effect Level (LOAEL) is the lowest exposure level used in the study that caused a harmful health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific end point used to

quantify the adverse effect accompanies the LOAEL. The "Less Serious" respiratory effect reported in key number 18 (hyperplasia) occurred at a LOAEL of 10 ppm.

(10) Reference The complete reference citation is given in Chapter 8 of the profile.

(11) CEL A Cancer Effect Level (CEL) is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiological studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses which did not cause a measurable increase in cancer.

(12) Footnotes Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. Footnote "c" indicates the ppm in key number 18 was used to derive an MRL of 0.005 ppm.

LEGEND

See LSE Figure 2-1

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure levels for particular exposure duration.

(13). Exposure Duration The same exposure periods appear as in the LSE table. In this example, health effects observed within the intermediate and chronic exposure periods are illustrated.

(14). Health Effect These are the categories of health effects for which reliable quantitative data exist. The same health effects appear in the LSE table.

(15). Levels of Exposure Exposure levels for each health effect in LSE tables are graphically displayed in the LSE figures. Exposure levels are reported on the log scale “y” axis. Inhalation exposure is reported in mg/m^3 ppm and oral exposure is reported in mg/kg/day.

(16). NOAEL In this example, 18r NOAEL is the critical end point for which an intermediate inhalation exposure MRL is based. As you can see from the LSE figure key, the open-circle symbol indicates a NOAEL for the test species (rat). The key number 18 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 3 ppm (see entry 18 in the Table) to the MRL of 0.005 ppm (see footnote "b" in the LSE table).

(17). CEL Key number 38r is one of three studies for which Cancer Effect Levels(CELs) were derived. The diamond symbol refers to a CEL for the test species (rat). The number 38 corresponds to the entry in e the LSE table.

APPENDIX A

(18). Estimated UDDer-Bound Human Cancer Risk Levels This is the range associated with the upper-bound for lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. These risk levels are derive.d from EPA's Human Health Assessment Group's upper-bound estimates of the slope of the cancer dose response curve at low dose levels (q_1*).

(19). Key to LSE Figure The Key explains the abbreviations and symbols used in the figure.

SAMPLE

1 → **TABLE 2-1. Levels of Significant Exposure to [Chemical x] - Inhalation**

Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
2 → INTERMEDIATE EXPOSURE							
3 → Systemic	5	6	7	8	9		10
4 → 18	Rat	13 wk 5d/wk 6hr/d	Resp	3[b]	10 (hyperplasia)		Nitschke et al. 1981
CHRONIC EXPOSURE							
Cancer						11	
38	Rat	18 mo 5d/wk 7hr/d				20 (CEL, multiple organs)	Wong et al. 1982
39	Rat	89-104 wk 5d/wk 6hr/d				10 (CEL, lung tumors, nasal tumors)	NTP 1982
40	Mouse	79-103 wk 5d/wk 6hr/d				10 (CEL, lung tumors, hemangiosarcomas)	NTP 1982

[a] The number corresponds to entries in Figure 2-1.

12 → [b] Used to derive an intermediate inhalation Minimal Risk Level (MRL) of 5×10^{-3} ppm; dose adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animal to humans, 10 for human variability).

CEL = cancer effect level; d = day(s); hr = hour(s); LOAEL = lowest-observed-adverse-effect level; mo = month(s); NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s)

SAMPLE

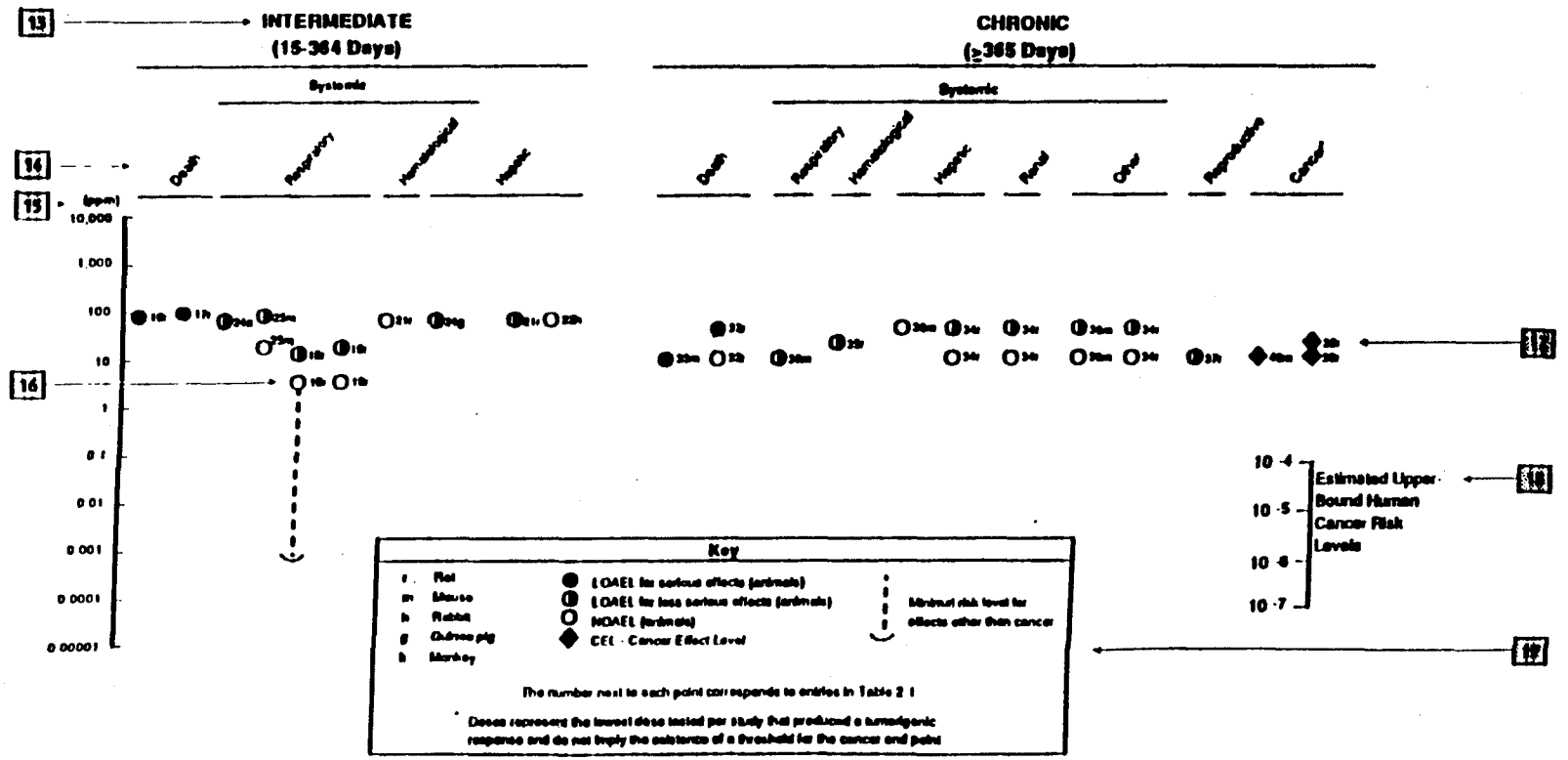

FIGURE 2-1. Levels of Significant Exposure to [Chemical X]-Inhalation

APPENDIX A

Chapter 2 (Section 2.4)

Relevance to Public Health

The Relevance to Public Health section provides a health effects summary based on evaluations of existing toxicological, epidemiological, and toxicokinetic information. This summary is designed to present interpretive, weight-of-evidence discussions for human health end points by addressing the following questions.

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

The section discusses health effects by end point. Human data are presented first, then animal data. Both are organized by route of exposure (inhalation, oral, and dermal) and by duration (acute, intermediate, and chronic). In vitro data and data from parenteral routes (intramuscular, intravenous, subcutaneous, etc.) are also considered in this section. If data are located in the scientific literature, a table of genotoxicity information is included.

The carcinogenic potential of the profiled substance is qualitatively evaluated, when appropriate, using existing toxicokinetic, genotoxic, and carcinogenic data. ATSDR does not currently assess cancer potency or perform cancer risk assessments. MRLs for noncancer end points if derived, and the end points from which they were derived are indicated and discussed in the appropriate section(s).

Limitations to existing scientific literature that prevent a satisfactory evaluation of the relevance to public health are identified in the Identification of Data Needs section.

Interpretation of Minimal Risk Levels

Where sufficient toxicologic information was available, MRLs were derived. MRLs are specific for route (inhalation or oral) and duration (acute, intermediate, or chronic) of exposure. Ideally, MRLs can be derived from all six exposure scenarios (e.g., Inhalation - acute, -intermediate, - chronic; Oral - acute, - intermediate, - chronic). These MRLs are not meant to support regulatory action, but to aquainthealth professionals with exposure levels at which adverse health effects are not expected to occur in humans. They should help physicians and public health officials determine the safety of a community living near a substance emission, given the concentration of a contaminant in air or the estimated daily dose received via food or water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

APPENDIX A

MRL users should be familiar with the toxicological information on which the number is based. Section 2.4, "Relevance to Public Health," contains basic information known about the substance. Other sections such as 2.6, "Interactions with Other Chemicals" and 2.7, "Populations that are Unusually Susceptible" provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology used by the Environmental Protection Agency (EPA) (Barnes and Dourson, 1988; EPA 1989a) to derive reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the end point which, in its best judgement, represents the most sensitive humanhealth effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential effects (e.g., systemic, neurological, and developmental). In order to compare NOAELs and LOAELs for specific end points, all inhalation exposure levels are adjusted for 24hr exposures and all intermittent exposures for inhalation and oral routes of intermediate and chronic duration are adjusted for continous exposure (i.e., 7 days/week). If the information and reliable quantitative data on the chosen end point are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest NOAEL that does not exceed any adverse effect levels. The NOAEL is the most suitable end point for deriving an MRL. When a NOAEL is not available, a Less Serious LOAEL can be used to derive an MRL, and an uncertainty factor (UF) of 10 is employed. MRLs are not derived from Serious LOAELs. Additional uncertainty factors of 10 each are used for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the adjusted inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a substance-specific MRL are provided in the footnotes of the LSE Tables.

APPENDIX B

ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
ADME	Absorption, Distribution, Metabolism, and Excretion
ATSDR	Agency for Toxic Substances and Disease Registry
BCF	bioconcentration factor
BSC	Board of Scientific Counselors
CDC	Centers for Disease Control
CEL	Cancer Effect Level
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
CLP	Contract Laboratory Program
cm	centimeter
CNS	central nervous system
DHEW	Department of Health, Education, and Welfare
DHHS	Department of Health and Human Services
DOL	Department of Labor
ECG	electrocardiogram
EEG	electroencephalogram
EPA	Environmental Protection Agency
EKG	see ECG
FAO	Food and Agricultural Organization of the United Nations
FEMA	Federal Emergency Management Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
f_1	first generation
fpm	feet per minute
ft	foot
FR	Federal Register
g	gram
GC	gas chromatography
HPLC	high performance liquid chromatography
hr	hour
IDLH	Immediately Dangerous to Life and Health
IARC	International Agency for Research on Cancer
ILO	International Labor Organization
in	inch
Kd	adsorption ratio
kg	kilogram
K_{oc}	octanol-soil partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{Lo}	lethal concentration low
LC_{50}	lethal concentration 50 percent kill
LD_{Lo}	lethal dose low
LD_{50}	lethal dose 50 percent kill
LOAEL	lowest-observed-adverse-effect level

APPENDIX B

LSE	Levels of Significant Exposure
m	meter
mg	milligram
min	minute
mL	milliliter
mm	millimeters
mmol	millimole
mppcf	millions of particles per cubic foot
MRL	Minimal Risk Level
MS	mass spectrometry
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NIOSHTIC	NIOSH's Computerized Information Retrieval System
nm	nanometer
ng	nanogram
NHANES	National Health and Nutrition Examination Survey
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NOES	National Occupational Exposure Survey
NOHS	National Occupational Hazard Survey
NPL	National Priorities List
NRC	National Research Council
NTIS	National Technical Information Service
NTP	National Toxicology Program
OSHA	Occupational Safety and Health Administration
PEL	permissible exposure limit
pg	picogram
pmol	picomole
PHS	Public Health Service
PMR	proportional mortality ratio
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
REL	recommended exposure limit
RfD	Reference Dose
RTECS	Registry of Toxic Effects of Chemical Substances
sec	second
SCE	sister chromatid exchange
SIC	Standard Industrial Classification
SMR	standard mortality ratio
STEL	short-term exposure limit
STORET	STORAGE and RETRIEVAL
TLV	threshold limit value
TSCA	Toxic Substances Control Act
TRI	Toxics Release Inventory
TWA	time-weighted average
U.S.	United States

APPENDIX B

UF	uncertainty factor
WHO	World Health Organization
$>$	greater than
$\geq$	greater than or equal to
$=$	equal to
$<$	less than
$\leq$	less than or equal to
%	percent
α	alpha
β	beta
δ	delta
γ	gamma
μm	micron
μg	microgram

APPENDIX C

PEER REVIEW

A peer review panel was assembled for 2-butanone. The panel consisted of the following members: Dr. Michael Norvell, Private Consultant, Ringoes, New Jersey; Dr. Rolf Hartung, Department of Environmental and Industrial Health, University of Michigan, Ann Arbor, Michigan; and Dr. Vincent Garry, Director, Laboratory for Environmental Medicine and Pathology, University of Minnesota, Minneapolis, Minnesota. These experts collectively have knowledge of 2-butanone's physical and chemical properties, toxicokinetics, key health end points, mechanisms of action, human and animal exposure, and quantification of risk to humans. All reviewers were selected in conformity with the conditions for peer review specified in the Comprehensive Environmental Response, Compensation, and Liability Act of 1986, Section 104.

Scientists from the Agency for Toxic Substances and Disease Registry (ATSDR) have reviewed the peer reviewers' comments and determined which comments will be included in the profile. A listing of the peer reviewers' comments not incorporated in the profile, with a brief explanation of the rationale for their exclusion, exists as part of the administrative record for this compound. A list of databases reviewed and a list of unpublished documents cited are also included in the administrative record.

The citation of the peer review panel should not be understood to imply its approval of the profile's final content. The responsibility for the content of this profile lies with the ATSDR.

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TOXICOLOGICAL PROFILE FOR CARBON DISULFIDE

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

August 1996

DISCLAIMER

The use of company or product name(s) is for identification only and does not imply endorsement by the Agency for Toxic Substances and Disease Registry.

UPDATE STATEMENT

A Toxicological Profile for Carbon Disulfide was released in September 1992. This edition supersedes any previously released draft or final profile.

Toxicological profiles are revised and republished as necessary, but no less than once every three years. For information regarding the update status of previously released profiles, contact ATSDR at:

Agency for Toxic Substances and Disease Registry
Division of Toxicology/Toxicology Information Branch
1600 Clifton Road NE, E-29
Atlanta, Georgia 30333

FOREWORD

This toxicological profile is prepared in accordance with guidelines* developed by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA). The original guidelines were published in the *Federal Register* on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for the hazardous substance described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a hazardous substance's toxicologic properties. Other pertinent literature is also presented, but is described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

The focus of the profiles is on health and toxicologic information; therefore, each toxicological profile begins with a public health statement that describes, in nontechnical language, a substance's relevant toxicological properties. Following the public health statement is information concerning levels of significant human exposure and, where known, significant health effects. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to protection of public health are identified by ATSDR and EPA.

Each profile includes the following:

(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a hazardous substance to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects;

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure that present a significant risk to human health of acute, subacute, and chronic health effects; and

(C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

The principal audience for the toxicological profiles is health professionals at the Federal, State, and local levels; interested private sector organizations and groups; and members of the public.

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed. Staff of the Centers for Disease Control and Prevention and other Federal scientists have also reviewed the profile. In addition, this profile has been peer-reviewed by a nongovernmental panel and was made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

David Satcher, M.D., Ph.D.
Administrator
Agency for Toxic Substances and
Disease Registry

*Legislative Background

The toxicological profiles are developed in response to the Superfund Amendments and Reauthorization Act (SARA) of 1986 (Public Law 99-499) which amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). This public law directed ATSDR to prepare toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. The availability of the revised priority list of 275 hazardous substances was announced in *the Federal Register* on April 29,1996 (61 FR 18744). For prior versions of the list of substances, see *Federal Register* notices dated April 17,1987 (52 FR 12866); October 20, 1988 (53 FR 41280); October 26,1989 (54 FR 43619); October 17,1990 (55 FR 42067); October 17, 1991 (56 FR 52166); October 28,1992 (57 FR 48801); and February 28,1994 (59 FR 9486). Section 104(I)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list.

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THE PROFILE HAS UNDERGONE THE FOLLOWING ATSDR INTERNAL REVIEWS:

1. Green Border Review. Green Border review assures the consistency with ATSDR policy.

2. Health Effects Review. The Health Effects Review Committee examines the health effects chapter of each profile for consistency and accuracy in interpreting health effects and classifying end points.

3. Minimal Risk Level Review. The Minimal Risk Level Workgroup considers issues relevant to substance-specific minimal risk levels (MRLs), reviews the health effects database of each profile, and makes recommendations for derivation of MRLs.

PEER REVIEW

A peer review panel was assembled for carbon disulfide. The panel consisted of the following members:

1. Dr. Henry Peters, Professor of Neurology, University of Wisconsin, Madison, WI
2. Dr. Shane Que Hee, Professor of Environmental Health Sciences, UCLA School of Public Health, Los Angeles, CA
3. Dr. Robert Rubin, Professor of Toxicology, Environmental Health Sciences, Johns Hopkins School of Hygiene and Public Health, Baltimore, MD

These experts collectively have knowledge of carbon disulfide's physical and chemical properties, toxicokinetics, key health end points, mechanisms of action, human and animal exposure, and quantification of risk to humans. All reviewers were selected in conformity with the conditions for peer review specified in Section 104(i)(l3) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

Scientists from the Agency for Toxic Substances and Disease Registry (ATSDR) have reviewed the peer reviewers' comments and determined which comments will be included in the profile. A listing of the peer reviewers' comments not incorporated in the profile, with a brief explanation of the rationale for their exclusion, exists as part of the administrative record for this compound. A list of databases reviewed and a list of unpublished documents cited are also included in the administrative record.

The citation of the peer review panel should not be understood to imply its approval of the profile's final content. The responsibility for the content of this profile lies with the ATSDR.

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1. PUBLIC HEALTH STATEMENT

This public health statement tells you about carbon disulfide and the effects of exposure.

The Environmental Protection Agency (EPA) identifies the most serious hazardous waste sites in the nation. These sites make up the National Priorities List (NPL) and are the sites targeted for long-term federal cleanup. Carbon disulfide has been found in at least 200 of the 1,430 current or former NPL sites. However, it's unknown how many NPL sites have been evaluated for this substance. As more sites are evaluated, the sites with carbon disulfide may increase. This information is important because exposure to this substance may harm you and because these sites may be sources of exposure.

When a substance is released from a large area, such as an industrial plant, or from a container, such as a drum or bottle, it enters the environment. This release does not always lead to exposure. You can be exposed to a substance only when you come into contact with it. You may be exposed by breathing, eating, or drinking the substances or by skin contact.

If you are exposed to carbon disulfide, many factors determine whether you'll be harmed. These factors include the dose (how much), the duration (how long), and how you come in contact with it. You must also consider the other chemicals you're exposed to and your age, sex, diet, family traits, life-style, and state of health.

1.1 WHAT IS CARBON DISULFIDE?

Pure carbon disulfide is a colorless liquid with a pleasant odor that smells sweet, The impure carbon disulfide that is usually used in most industrial processes, however, is a yellowish liquid with an unpleasant odor like that of rotting radishes. Carbon disulfide evaporates at room temperature, and the vapor is more than twice as heavy as air. Carbon disulfide easily explodes in air and also catches fire very easily.

In nature, small amounts of carbon disulfide are found in gases released to the earth's surface, for example, in volcanic eruptions or over marshes. Microorganisms in the soil can also produce gas containing carbon disulfide. Commercial carbon disulfide is made by combining carbon and sulfur at very high temperatures. Several industries use carbon disulfide as a raw material to make such things as rayon, cellophane, and carbon tetrachloride. Currently, the largest user of this chemical is the viscose rayon industry. Carbon disulfide is also used to dissolve rubber to produce tires and as a raw material to make some pesticides. See Chapters 3, 4, and 5 for more information on the chemical and physical properties, use, and environmental fate of carbon disulfide.

1.2 WHAT HAPPENS TO CARBON DISULFIDE WHEN IT ENTERS THE ENVIRONMENT?

Carbon disulfide evaporates rapidly when released to the environment. The amount of carbon disulfide released into the air through natural processes is difficult to judge because it is in such small amounts in nature. This also makes it hard to monitor carbon disulfide and to explain how it behaves when it comes into contact with other compounds. Most carbon disulfide in the air and in surface water is from manufacturing and processing activities. However, it is found naturally in coastal and ocean waters. Carbon disulfide has also been found in the groundwater and soil at some EPA research sites around the country, but the number of research sites that have carbon disulfide is small.

Once released to the environment, carbon disulfide moves quickly to the air. Once in the air, carbon disulfide stays close to the ground because it is heavier than the surrounding air. It is estimated that carbon disulfide will break down into simpler components after approximately 12 days. Carbon disulfide moves through soils fairly quickly. Carbon disulfide accidentally released to soils normally evaporates rapidly. However, since carbon disulfide does not bind tightly to soils, the amount that does not evaporate can easily move down through the soil into groundwater. Since it is very mobile, it is not likely to stay in the soil long enough to be broken down. It does not remain very long in water either because it evaporates within minutes. However, if dissolved in water, it is relatively stable and is not easily broken down.

It is estimated that carbon disulfide is not taken up in significant amounts by the organisms living in water.

1.3 HOW MIGHT I BE EXPOSED TO CARBON DISULFIDE?

Carbon disulfide can enter your body if you breath air, drink water, or eat foods that contain it. You can also be exposed by skin contact with soil, water, or other substances that contain it. Oceans are a major natural source. The amount of carbon disulfide found in the air from natural sources such as volcanoes is so low that good measurements are not available from many areas. One measurement shows that carbon disulfide produced by marshes contributes less than 8% of the sulfur in the upper atmosphere.

Small amounts of carbon disulfide can enter the air by evaporation and as a by-product of several manufacturing processes. It is not clear how long carbon disulfide stays in the air. Estimates range from 1 to 10 weeks. The people most often exposed to carbon disulfide are workers in plants that use carbon disulfide in their manufacturing processes. The main way they are exposed is through the air, and secondarily the skin. Carbon disulfide has also been found in small amounts in some drinking water in the United States. Chapter 5 contains more information on how you might be exposed to carbon disulfide.

1.4 HOW CAN CARBON DISULFIDE ENTER AND LEAVE MY BODY?

Most people are exposed to carbon disulfide by breathing air that contains it. Carbon disulfide easily and rapidly enters your bloodstream through the lungs. Carbon disulfide can also enter your body through your skin, or by eating or drinking foods that are contaminated with the chemical. About l0-30% of carbon disulfide that the body absorbs leaves through the lungs; less than 1% leaves in the urine. The rest of the absorbed carbon disulfide (70-90%) is changed in the body and leaves the body in the urine in the form of other chemicals. Small amounts of carbon disulfide also leave the body in sweat and saliva. For more information, see Chapter 2.

1.5 HOW CAN CARBON DISULFIDE AFFECT MY HEALTH?

At very high levels (10,000 parts of carbon disulfide per million parts [ppm] of air), carbon disulfide may be life threatening because of its effects on the nervous system. Studies in animals show that high levels of carbon disulfide can damage the heart. People who breathed carbon disulfide near an accident involving a railroad car showed changes in breathing and some chest pains. Among workers who breathed about 8 ppm, some developed very slight changes in their nerves. Some workers who breathed more than 20 ppm during working hours for at least 6 months had headaches, tiredness, and trouble sleeping. However, the workers may have been exposed to other chemicals besides carbon disulfide. The current standard for exposure in the workplace is 20 ppm over an 8-hour day and a 5-day work week.

Studies in animals indicate that carbon disulfide can affect the normal functions of the brain, liver, and heart. However, the amount of carbon disulfide in the air to which animals in these studies were exposed was much higher than the amounts in the air that the general public usually breathes. The brains, livers, and hearts of the animals were affected only after breathing air that contained carbon disulfide for days, months, or years. After pregnant rats breathed 225 ppm carbon disulfide in the air, some of the newborn rats died or had birth defects.

There is no information on health effects in people who eat food or drink water contaminated with carbon disulfide. Animals fed food that contained carbon disulfide developed liver and heart disease, and some showed abnormal behavior. These amounts, however, were very much higher than those that occur in drinking water supplies. When pregnant animals received large doses of carbon disulfide in their diet, some of the newborns died or had birth defects.

Skin contact with spilt carbon disulfide can lead to burns at the contact site. In studies that examined the harmful effects of skin contact with carbon disulfide, workers in a rayon plant who handled fibers made with carbon disulfide for more than 14 days developed blisters on their fingers. Rabbits developed blisters and ulcers on the treated areas of their ears.

1.6 IS THERE A MEDICAL TEST TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO CARBON DISULFIDE?

Carbon disulfide itself can be measured in breath, urine, and blood. It breaks down in the body into other chemical substances called metabolites. These substances can be found and measured in the urine. After carbon disulfide enters your body, these substances reach higher levels than normally found. One chemical test using urine can be done to tell whether the levels of these breakdown substances from carbon disulfide are higher than normal. This test requires special equipment and is not routinely available in a doctor's office. The test is not specific for carbon disulfide exposure because other chemicals can also produce these metabolites. Therefore, it cannot be used to find out exactly how much carbon disulfide you were exposed to or to predict whether you'll be harmed. Also, the test can only be used if you have breathed in at least 16 ppm; this test can be used for determining longer term exposure to carbon disulfide. A second test based on a specific metabolite is more sensitive and specific. It also requires special equipment and cannot tell you exactly how much carbon disulfide you were exposed to or predict whether you'll be harmed. Carbon disulfide leaves the body quickly in the breath and in the urine. See chapters 2 and 6 for more information on testing for carbon disulfide.

1.7 WHAT RECOMMENDATIONS HAS THE FEDERAL GOVERNMENT MADE TO PROTECT HUMAN HEALTH?

The federal government has set regulations to protect individuals from the possible health effects of eating, drinking, or breathing carbon disulfide. The EPA suggested that taking into your body each day an amount equal to 0.1 mg (milligram) of carbon disulfide per kg (kilogram) of your body weight is not likely to cause any significant (noncancer) harmful health effects.

The Occupational Safety and Health Administration (OSHA) regulates levels of carbon disulfide in the workplace (see Table 7-l). OSHA requires that workroom air contain no

more than an average of 20 ppm of carbon disulfide over an 8-hour working shift for 5 consecutive days in a work week.

The National Institute for Occupational Safety and Health (NIOSH) recommends that the average workroom air levels of carbon disulfide not exceed 1 ppm over a l0-hour period. For more information on rules and standards for carbon disulfide, see Chapter 7.

1.8 WHERE CAN I GET MORE INFORMATION?

If you have any more questions or concerns, please contact your community or state health or environmental quality department or

Agency for Toxic Substances and Disease Registry
Division of Toxicology
1600 Clifton Road NE, Mailstop E-29
Atlanta, Georgia 30333
(404) 639-6000

This agency can also provide you with the location of the occupational and environmental health clinics. These clinics specialize in the recognition, evaluation, and treatment of illness resulting from exposure to hazardous substances.

2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of carbon disulfide. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure - inhalation, oral, and dermal; and then by health effect - death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods - acute (14 days or less), intermediate (15-364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest observed- adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into “less serious” or “serious” effects. “Serious” effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). “Less serious” effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, “less serious” LOAEL, or “serious” LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify

these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between “less serious” and “serious” effects. The distinction between “less serious” effects and “serious” effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user’s perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals or exposure levels below which no adverse effects have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for carbon disulfide. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1989b), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

A User's Guide has been provided at the end of this profile (see Appendix A). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

2.2.1 Inhalation Exposure

Carbon disulfide can exist in air as vapor. Table 2-l and Figure 2-l summarize the available quantitative information on the health effects that have been observed in humans and animals following inhalation exposure to carbon disulfide. All exposure levels are expressed as parts per million (ppm). In many workplace exposures, exposure could be by inhalation and skin exposure, rather than inhalation exposure alone.

2.2.1.1 Death

Several epidemiology studies have reported increased mortality among workers in viscose rayon plants who were occupationally exposed to carbon disulfide as well as other chemicals (Hernberg et al. 1970, 1973; Tolonen et al. 1975, 1979). Deaths have also been reported in a community in India following an accidental release of large amounts of carbon disulfide, hydrogen sulfide, and sulfuric acid from a viscose rayon plant (Kamat 1994). However, no definitive or consistent conclusions can be drawn from these studies because of concomitant exposure to other chemicals, uncertainty about exposure concentrations, and the likelihood of multiple routes of exposure.

In a 10-year (1975-1985) epidemiological study of 251 workers exposed to carbon disulfide and 124 controls in two viscose rayon factories in Czechoslovakia, increases in total and cardiovascular mortality were noted in spinners exposed to high levels of carbon disulfide (Balcarova and Halik 1991). Although associated levels of exposure were not quantified for this particular group, the study authors estimate that exposures ranged from less than 9.6 to 48 ppm. However, insufficient data were provided to fully support their conclusions. An approximately 15% increase in deaths resulting from circulatory disease was observed among Dutch viscose rayon workers exposed to carbon disulfide concentrations which were described as "at least 7 ppm, and possibly higher" (Swaen et al. 1994). The increased risk of dying from circulatory disease was greatest 20-30 years after the start of the exposure.

The approximate LC_{50} for a 60-minute exposure in male mice was 220 ppm (Gibson and Roberts 1972). Inhalation exposure of pregnant rats to 642 ppm during gestation produced 33% mortality among dams; exposure to 225 ppm produced 35% mortality among pups (Lehotsky et al. 1985). Four of 22 mice died following inhalation exposure to 800 ppm for 6 hours a day, 5 days a week, for 90 days (Toxigenics 1983c). The reliable LC_{50} values for male mice and the LOAEL value for female rats for the acute-duration category are recorded in Table 2-l and plotted in Figure 2-l.

2.2.1.2 Systemic Effects

No studies were located regarding musculoskeletal and dermal effects in humans or animals after inhalation exposure to carbon disulfide. Information on respiratory, cardiovascular, gastrointestinal, hematological, hepatic, renal, endocrine, ocular, body weight, and other systemic effects after inhalation exposure is presented below. The highest NOAEL and all LOAEL values from each reliable study for these systemic effects in each species and each duration category are recorded in Table 2-l and plotted in Figure 2-l.

Respiratory Effects. Following an accident involving a railroad car, 27 individuals were exposed via inhalation to an unspecified concentration of carbon disulfide. Subtle and transient changes in pulmonary function were manifested as reduced vital capacity and decreased partial pressure of arterial oxygen (Spyker et al. 1982). Dyspnea was reported in 77 of the 123 persons following an accidental release of large amounts of carbon disulfide, hydrogen sulfide, and sulfuric acid from a viscose rayon plant in India (Kamat 1994). Exposure concentrations were not stated.

White male Wistar rats exposed to 803 ppm carbon disulfide for 18 hours showed reduced cardiac and respiratory rates and severe narcosis (Tarkowski and Sobczak 1971). However, this study used only six or seven animals and only one dose was tested.

Cardiovascular Effects. In humans, vascular atherosclerotic changes are a primary effect following long-term exposure to carbon disulfide. This is supported by epidemiological studies that have established a relationship between occupational exposure to carbon disulfide and increased mortality due to coronary heart disease (Hernberg et al. 1970, 1971, 1973; MacMahon and Monson 1988; Tiller et al. 1968; Tolonen et al. 1979) and circulatory disease deaths (Swaen et al. 1994). Milder manifestations such as angina have also been documented (Hernberg et al. 1971; Tolonen et al. 1979).

TABLE 2-1. Levels of Significant Exposure to Carbon Disulfide - Inhalation

Key to[a] figure	Species (strain)	Exposure duration/ frequency	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
ACUTE EXPOSURE							
Death							
1	Rat (CFY)	8 d 6hr/d Gd 7-15				642 F (33% mortality)	Lehotsky et al. 1985
2	Mouse (Swiss-Webster)	60 min				220 M (LC_{50})	Gibson and Roberts 1972
Systemic							
3	Rat (Wistar)	8 hr	Hepatic		20 F (increase in total lipids)		Freundt et al. 1974a
4	Rat (Wistar)	18 hrs	Resp			803 M (decreased respiratory rate)	Tarkowski and Sobczak 1971
			Cardio			803 M (decreased cardiac rate)	
5	Rabbit	12 d 6hr/d	Hemato	1100			Brieger 1949
Neurological							
6	Rat (Wistar)	1 hr			642 M (significant decrease in brain noardrenaline; increased sensitivity to amphetamine)		Magos et al. 1974
7	Rat (Wistar)	18 hr				803 M (severe narcosis, straightening of hindlimbs)	Tarkowski and Sobczak 1971
8	Rat (Wistar)	12 hr			777.1 F (swollen mitochondria in brain; increased ATP)		Tarkowski et al. 1980.

TABLE 2-1. Levels of Significant Exposure to Carbon Disulfide - Inhalation (continued)

Key to[a] figure	Species (strain)	Exposure duration/ frequency	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
Developmental							
9	Rabbit (New Zealand White)	13 d 6hr/d Gd 6-18		300 F		600 F (increased post-implantation loss)	PAI 1991
INTERMEDIATE EXPOSURE							
Death							
10	Mouse (B6C3F1)	90 d 5d/wk 6hr/d				800 (4/22 died)	Toxigenics 1983c
Systemic							
11	Rat (NS)	1-6 mo 5d/wk 5-8 hr/d	Cardio	3.2 M		16 M (myocardial edema and microhemorrhages)	Antov et al. 1985
12	Rat (Long- Evans)	11 wk 7d/wk 7hr/d	Bd Wt	400 F	800 F (15% decrease body weight)		Rebert and Becker 1986
13	Rat (Sprague-Dawley)	15 d 6hr/d Gd 6-20	Bd Wt	200 F	400 F (19% decreased in maternal body weight gain)		Saillenfait et al. 1989
14	Rat (Wistar)	1-14 mo 5hr/d	Bd Wt		482 (decreased body weight)		Szendzikowski et al. 1974
15	Rat (Long- Evans)	10 wk 5d/wk 5hr/wk	Bd Wt	600 M			Tepe and Zenick 1984

TABLE 2-1. Levels of Significant Exposure to Carbon Disulfide - Inhalation (continued)

Key to[a] figure	Species (strain)	Exposure duration/ frequency	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
16	Rat (Wistar)	8 mo 6d/wk 5hr/d	Hepatic		74 (increased serum lipids)		Wronska-Nofer 1973
					161 (increased liver cholesterol synthesis)		
			Bd Wt	321		546 (26% decrease in body weight)	
17	Mouse (B6C3F1)	90 d 5d/wk 6hr/d	Hemato	300	800 (decreased RBC count, total hemoglobin, and hematocrit)		Toxigenics 1983c
			Hepatic	800			
			Renal	300		800 (nephropathy)	
			Ocular	800			
			Bd Wt	300	800 (decreased body weight 10-11%)		
	Neurological						
18	Monkey (Macaque)	5-13 wk 5d/wk 6hr/d				256 F (severely reduced visual acuity and contrast sensitivity due to effect on optic nerve; retinal ganglion cell degeneration)	Merigan et al. 1988
19	Rat (Long- Evans)	5 or 12 wk 5d/wk 6h/d			500 M (decrease in auditory startle reflex amplitude)		Clerici and Fechter 1991
20	Rat (Sprague-Dawley males, F344 females)	90 d 6h/d 5d/wk		50		300 (occasional swelling of axons in lumbar spinal cord)	Gottfried et al 1985

TABLE 2-1. Levels of Significant Exposure to Carbon Disulfide - Inhalation (continued)

Key to[a] figure	Species (strain)	Exposure duration/ frequency	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
21	Rat (Wistar)	1-14 mo 5hr/d				482 (axonal swelling and distended mitochondria)	Szendzikowski et al 1974
22	Rat (Wistar)	10 mo 6d/wk 5hr/d				578 M (loss of motor equilibrium, muscular weakness, hindlimb paresis)	Tarkowski and Sobczak 1971
23	Rat (Wistar)	10 mo 5hr/d 5d/wk			257 F (uncoupling of oxidative phosphorylation)		Tarkowski et al. 1980
24	Rat (Fischer- 344)	6 wk 5d/wk 4hr/d			642.2 M (decreased hindlimb extensor responses and motor coordination)		Tilson et al. 1979
25	Rat	8 mo 6d/wk 5hr/d		321		546 (paralysis of hindlimbs)	Wronska-Nofer 1973
26	Mouse	90 d 5d/wk 6hr/d		300		800 (degeneration of peripheral nerves)	Toxigenics 1983c
	Reproductive						
27	Rat (Long- Evans)	10 wk 5d/wk 6 hr/d		350 M		600 M (reduced plasma testosterone; slightly lower sperm counts)	Tepe and Zenick 1984
	Developmental						
28	Rat (Wistar, Sprague-Dawley)	19 d 6-7hr/d Gd 1-19		40 F			Hardin et al. 1981

TABLE 2-1. Levels of Significant Exposure to Carbon Disulfide - Inhalation (continued)

Key to[a] figure	Species (strain)	Exposure duration/ frequency	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
29	Rat (Sprague-Dawley)	Pregestation 3 wk 5d/wk 7hr/d, Gd 6-18 or 0-18		40 F			NIOSH 1980
30	Rat (Sprague-Dawley)	15 d 6hr/d Gd 6-20		200	400 (reduced fetal weight)		Saillenfait et al. 1989
31	Rabbit (New Zealand White)	24 d 6-7hr/d Gd 1-24		40 F			Hardin et al 1981
32	Rabbit (New Zealand White)	Pregestation 3 wk 5d/wk Gd 0-21 or Gd 7-21 7 hr/d		40 F			NIOSH 1980
CHRONIC EXPOSURE							
Systemic							
33	Human	3-12 yr	Cardio	9.6			Cirla and Graziano 1981
			Hemato	9.6			
Neurological							
34	Human	3-12 yr occup		9.6			Cirla and Graziano 1981

TABLE 2-1. Levels of Significant Exposure to Carbon Disulfide - Inhalation (continued)

Key to[a] figure	Species (strain)	Exposure duration/ frequency	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
35	Human	12.1 yr occup			7.6 [b] M (decreased peroneal nerve MCV and sural nerve SVC)		Johnson et al. 1983

[a]The number corresponds to entries in Figure 2-1.
[b]Used to derive a chronic inhalation Minimal Risk Level (MRL) of 0.3 ppm for carbon disulfide; the LOAEL of 7.6 ppm was divided by an uncertainty factor of 30 (10 for human variability and 3 for use of a minimal LOAEL).

ATP = adenosine triphosphate; Bd Wt = body weight; Cardio = cardiovascular; d = day(s); F = female; Gd = gestational day; Hemato = hematological; hr = hour(s); LC_{50} = lethal concentration, 50% kill; LOAEL = lowest-observed-adverse-effect level; M= male; MCV = motor nerve conduction velocity; min = minute(s); mo = month(s); NOAEL = no-observed-adverse-effect level; NS = not specified; occup = occupational; RBC = red blood cell; Resp = respiratory; SVC = sensory conduction velocity; wk = week(s); yr = year(s)

Figure 2-1. Levels of Significant Exposure to Carbon Disulfide – Inhalation

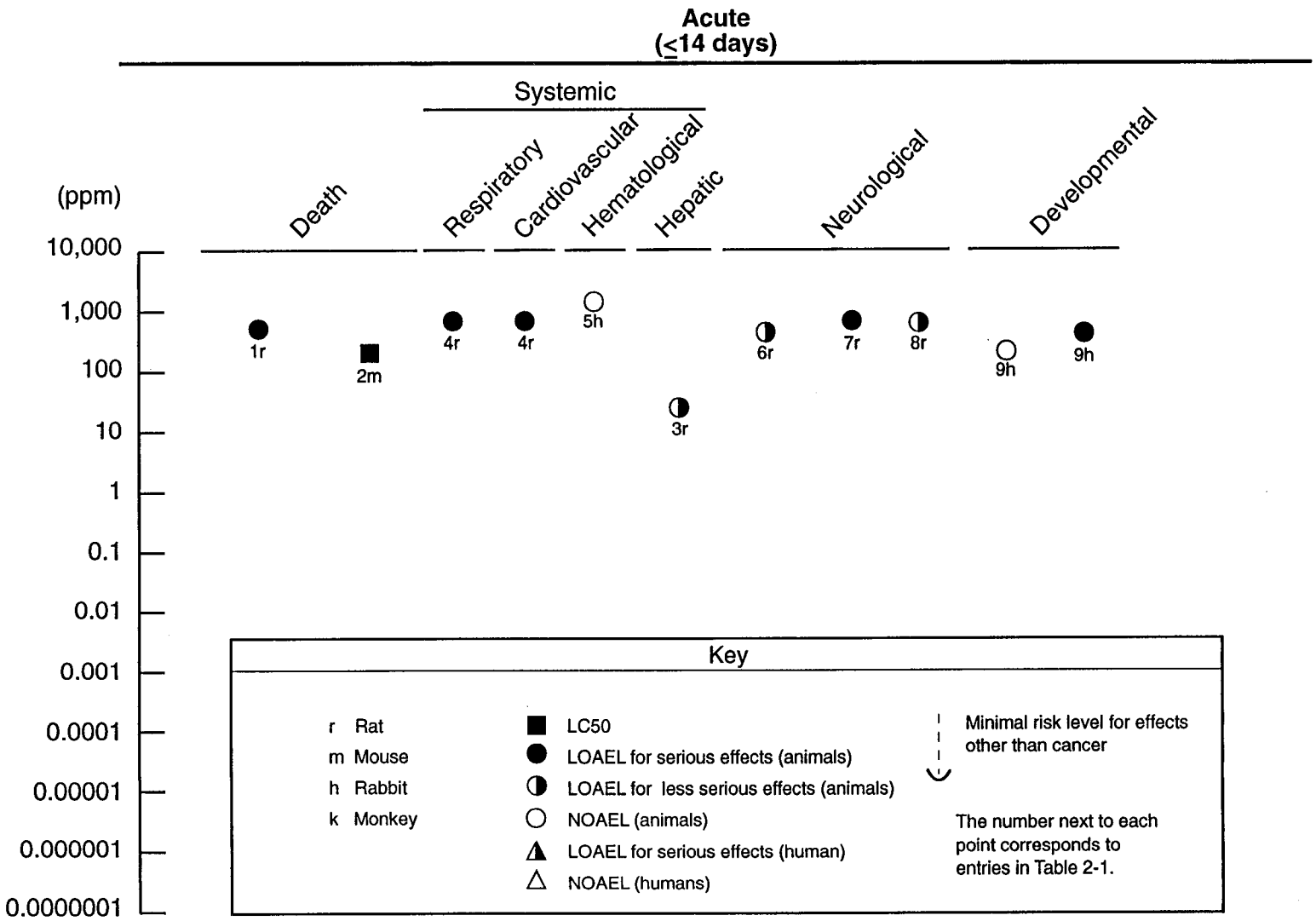


Figure 2-1. Levels of Significant Exposure to Carbon Disulfide – Inhalation (continued)

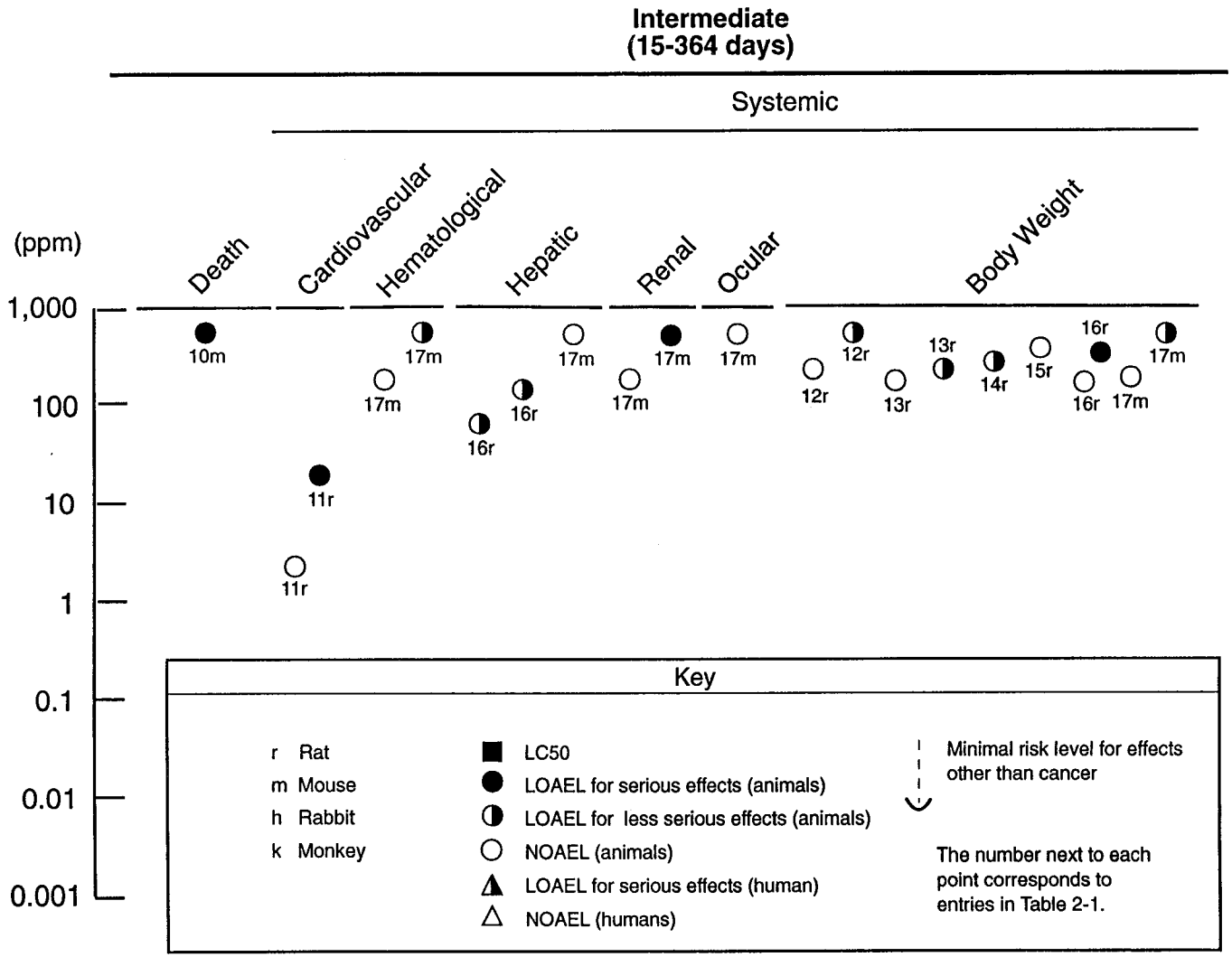


Figure 2-1. Levels of Significant Exposure to Carbon Disulfide – Inhalation (continued)

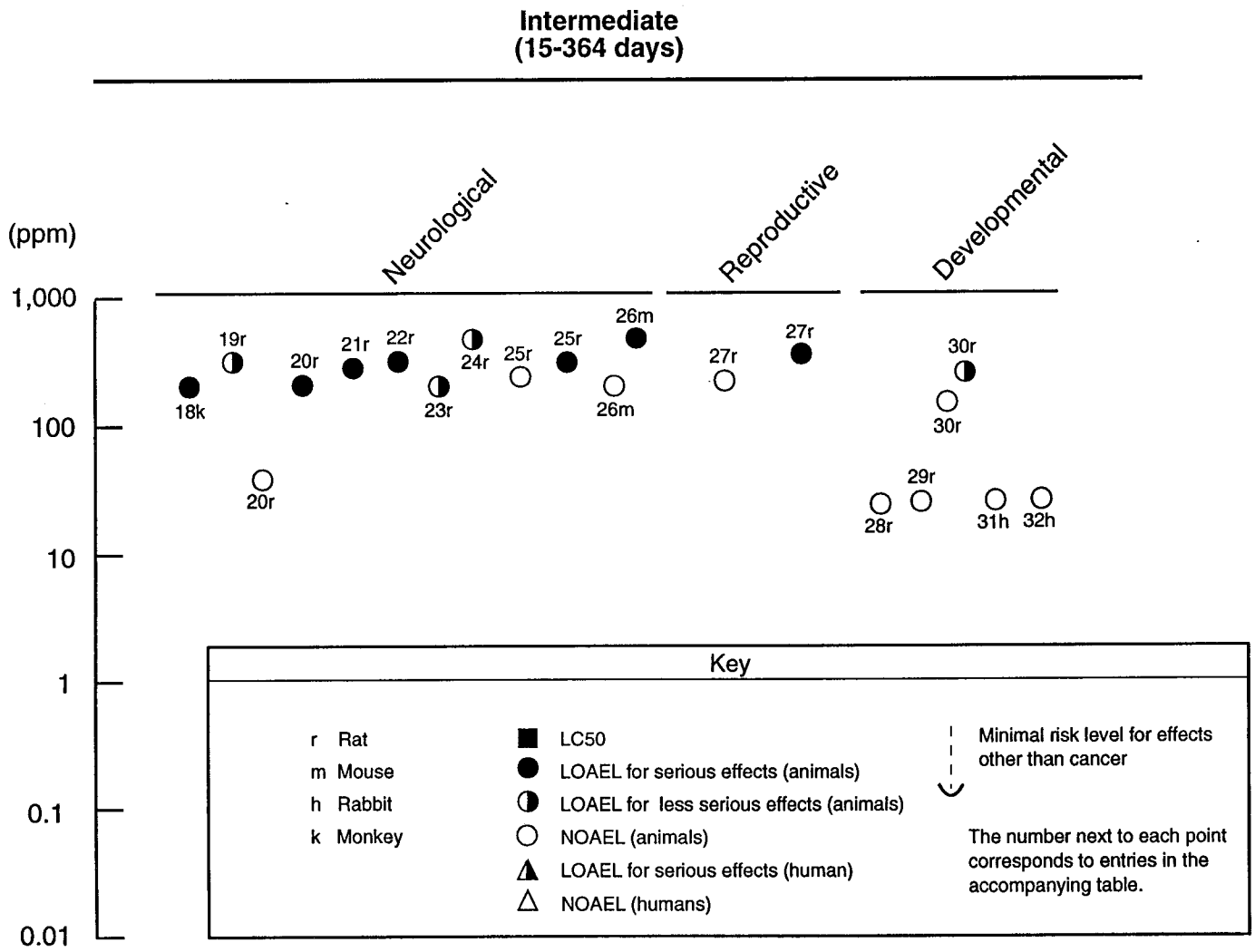
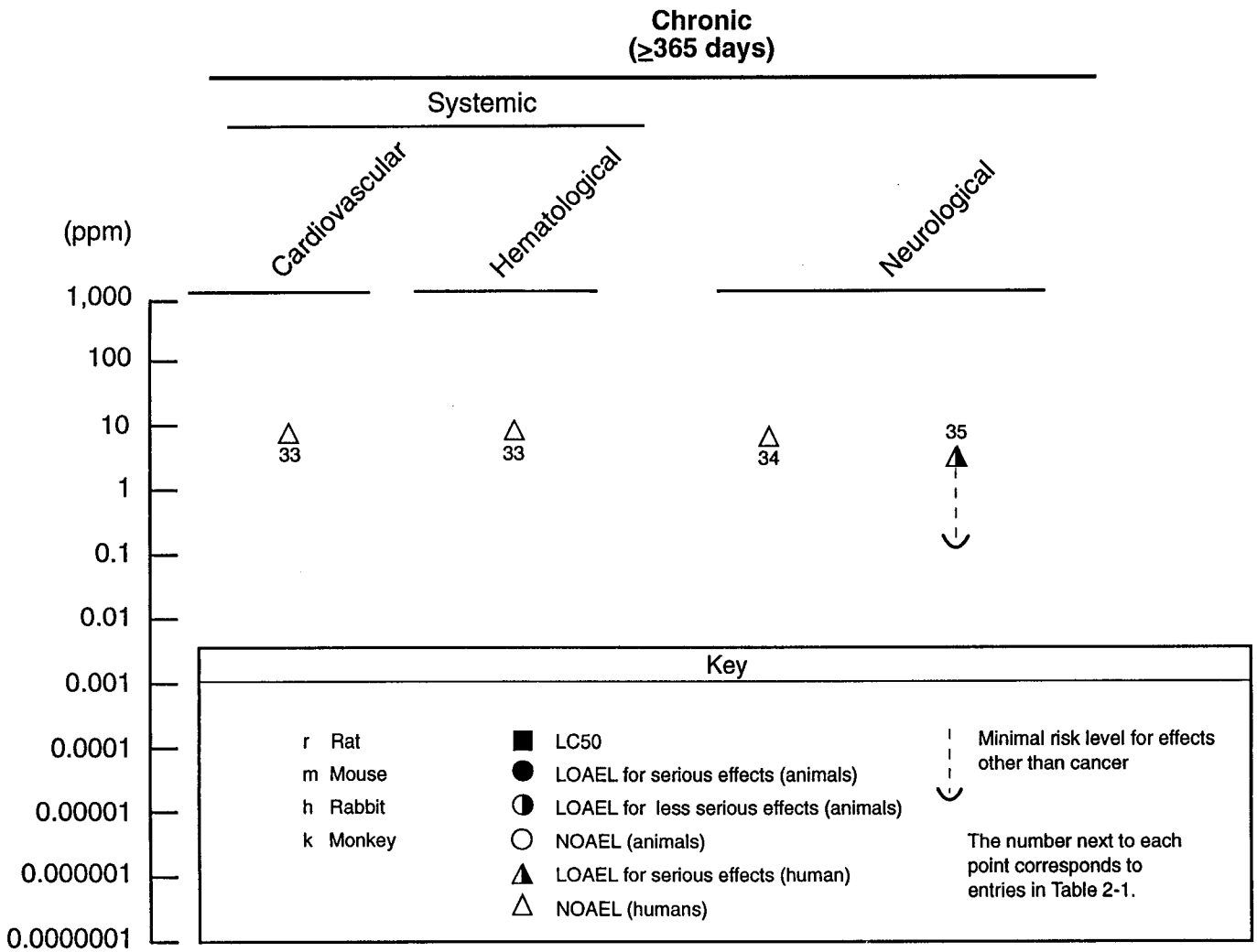


Figure 2-1. Levels of Significant Exposure to Carbon Disulfide – Inhalation (continued)



However, since reliable data on exposure levels were not available, it is impossible to establish a dose-response relationship or a NOAEL. In addition, coronary heart disease has a multicausal origin that is in part related to the saturated fat intake of the population and is also influenced by a large number of other risk factors such as smoking, other dietary habits, diabetes, and physical inactivity. A combination of two or more risk factors greatly increases the incidence of coronary heart disease, and therefore carbon disulfide may be a cofactor in the presence of other risk factors (WHO 1979). Another limitation with occupational studies reported from the viscose rayon industry is concurrent exposure to other chemicals such as hydrogen sulfide (Hernberg et al. 1970; Rubin and Arieff 1945; Swaen et al. 1994; Tolonen et al. 1979).

A retrospective mortality study revealed that 223 viscose rayon process workers employed for more than 10 years and exposed to carbon disulfide at concentrations in excess of 20 ppm had a statistically significant increase (2.5-fold) in deaths due to coronary heart disease from 1933 to 1962, compared to 174 nonprocess workers from the same factory (Tiller et al. 1968). Over the 30-year study period, 42% of all deaths in rayon process workers were attributed to coronary heart disease; the proportion was 24% for other rayon workers and 17% for other local males used as controls. The excess mortality was more pronounced in the 1940s and declined towards 1960, indicating a strong dependence on the intensity of exposure, which had decreased during this interval. The same study demonstrated that the death rate from coronary heart disease was proportionally higher among workers engaged in the viscose spinning process than in other workers. However, nonexposed workers also had a significantly higher death rate than expected for coronary heart disease, as did controls not employed in the viscose industry. These factors limit the value of this study. Other limitations include an inappropriately selected control group, failure to control for other coronary heart disease risk factors such as smoking, dietary habits, physical inactivity, and obesity, and failure to monitor blood pressure and blood lipid levels. In addition, there may have been concomitant exposures to other chemicals in these industrial environments.

A prospective mortality study at a Finnish plant during the period of 1967-1977 revealed a similar excess of deaths (2.5-fold) due to coronary heart disease (Tolonen et al. 1979). Two cohorts were followed over a 10-year period, 1967-1977; 343 viscose rayon workers exposed to carbon disulfide were individually matched with workers from the local paper mill. There was no significant difference between the exposed and control groups with regard to smoking habits, physical activity, obesity, or drug treatment. Carbon disulfide concentrations in workplace air were 10-30 ppm during the 1960s

20-60 ppm during the 1950s and higher in earlier time periods. The incidence of mortality due to coronary heart disease was 29/343 in the exposed group versus 11/343 in the control group. Periodic health surveys during the study revealed an increased incidence of angina and increased blood pressure compared to a well-matched control group (Tolonen et al. 1979). The incidence of deaths from coronary heart disease appeared to be much greater during the first 5 years as reported in interim results of the same cohorts, but the numbers were too small to draw any conclusions (Hernberg et al. 1970, 1973).

Increased mortality from cardiovascular causes was noted in a 1975-1985 epidemiological study of 251 workers exposed to carbon disulfide compared to 124 nonexposed workers in two viscose rayon factories in Czechoslovakia (Balcarova and Halik 1991). The workers (spinners) were exposed to “high” levels of carbon disulfide with estimated concentrations ranging from less than 9.6 to 48 ppm. An increased incidence of myocardial infarction was also noted in the highly exposed group compared to controls. However, this study should be interpreted with caution since scanty data were provided regarding methods employed.

In a study conducted on Egyptian workers employed in a viscose rayon factory, Kamal et al. (1991) found a significantly higher prevalence of pathological changes revealed by electrocardiogram (ECG). The study was conducted on 253 workers exposed to 20-45 ppm of carbon disulfide for 4-29 years; the control group consisted of 99 workers. No association was found between the duration of exposure and ECG activity; this finding indicates that the duration of exposure to carbon disulfide may not be a major risk factor unless there are other predisposing factors. The study was limited, however, because the exposure concentrations documented in factory records may not have been representative of the actual exposures.

Among men who had been exposed to carbon disulfide for 5 or more years between 1942 and 1967, the incidence of angina was 25% compared to 13% in unexposed controls, and a significant increase in blood pressure was seen (Hernberg et al. 1971, 1976; Tolonen et al. 1975). Nonfatal first cardiac infarctions were more frequent in the exposed group (11) than in the control group (4). The relative risk of a fatal myocardial infarction was 4.8 times greater among those exposed to carbon disulfide; 16 of 343 men died of coronary heart disease within the 5-year period compared to 3 of 343 men in the control group ($p<0.007$) (Hernberg et al. 1973; Tolonen et al. 1975). In a subsequent study, the original relative risk estimates were adjusted for potential confounding effects of hypertension and

aging. After these adjustments, carbon disulfide exposure yielded a relative risk of 2.3 for coronary disease mortality (Nurminen et al. 1982). Thus, although the prognosis for exposed workers was better with improved occupational hygiene and with a reduction in the length of exposure over a lifetime, there was apparently some increased risk attributable to carbon disulfide exposure in this cohort. However, there were no adjustments for possible concomitant exposures to other chemicals.

A follow-up study of 343 Finnish viscose rayon workers was performed to examine the incidence of cardiovascular mortality from 1967 to 1982 (Nurminen and Hernberg 1985). Exposure to carbon disulfide varied greatly (approximately 22 ppm to <l0 ppm), with a decrease in exposures after 1972. Within the first 5 years of follow-up (1967-1972), there was a 4.7-fold increase in ischemic and heart disease mortality compared with a cohort of paper mill workers. In the period of 1972-1974, the relative risk ratio was 3.2. After all workers with high coronary risk factors were removed from exposure (19% of the cohort was exposed in 1977 compared to 53% in 1972), the risk of cardiovascular death was reduced to a ratio of 1.0 in the years 1974-1982. This study indicates that the cardiotoxic effects of carbon disulfide may be reversible with removal of individuals from the toxic environment. Caution must be used in interpreting these data because of the increase in the incidence of cardiovascular events in the aging cohort population and the possibility that carbon disulfide accelerates death in high-risk individuals.

In a study of Japanese viscose rayon workers, no effects were noted on blood pressure or on the incidence of angina. The exposed group comprised 420 rayon filament workers; 390 controls were obtained from a local cuprammonium rayon factory (Sugimoto et al. 1978). Mean carbon disulfide air levels were below 20 ppm at the time of the study (about 1975); these levels had been higher (15-30 ppm) during the 1950s. These observations suggest that ethnic variation or other demographic factors (e.g., dietary habits) may affect the response to carbon disulfide. Cardiovascular effects on 50 viscose rayon workers occupationally exposed to an average range of 3.2-9.6 ppm carbon disulfide for 3-12 years were compared to a pair-matched control group from unexposed departments of the plant (Cirla and Graziano 1981). On ophthalmoscopy, two control and two exposed workers suffered minor vascular changes. Both systolic and diastolic mean blood pressures were higher in the control group. Differences were not statistically significant ($p \geq 0.10$). The use of a questionnaire, confirmed by electrocardiography, determined one case of arrhythmia and one case of coronary heart disease in the exposed group, with no cases reported for the control group. The one case of coronary heart disease suggests there is some cardiac effect of carbon disulfide, but no general conclusions can be reached by

this study. The results further suggest that occupational exposure below 9.6 ppm (maximum exposure concentration) for up to 12 years does not cause recognizable health damage. This interpretation must be viewed with caution because the maximum exposure duration to carbon disulfide was only 12 years, with most workers between 6 and 9 years at risk. In addition, workers were exposed to mean average concentrations of 3.2-8 ppm carbon disulfide. If carbon disulfide exerts effects through an arteriosclerotic process, this duration of exposure may not have been adequate to observe cardiovascular effects. Also, the study does not include the turnover rate due to death or attrition among workers, some of which could be attributable to cardiovascular effects.

In another study, the effects of carbon disulfide on the cardiovascular system of 1,498 rayon workers were evaluated in comparison to 481 acetate workers (Lieben et al. 1974). An electrocardiogram, blood pressure level, total cholesterol level, and occupational exposure history were obtained for each worker. The only statistically significant finding was a higher average blood pressure level (140/87) in workers in the rayon plants than in workers in the acetate plants (135/83). However, blood pressure readings did not differ significantly among workers exposed to high, medium, and low concentrations of carbon disulfide, which suggests the lack of a dose response. Although no data were presented, there could have been confounding exposures to other chemicals. Increased retinal arterial pressure but not brachial arterial pressure was observed in viscose rayon workers exposed to carbon disulfide at average concentrations of 64-161 ppm with peaks of 289 ppm for l-9 years (Maugeri et al. 1967). A correlation between effects and duration of exposure was not detected.

Physical examinations were completed both before and after 114 workers were exposed to carbon disulfide for 5 years (Chrostek-Maj and Czeczotko 1995a). Cardiovascular effects, as assessed by blood pressure and electrocardiograms, were not observed. Exposure concentrations were stated as a mean of 0-21 ppm and a median of 0-l.1 ppm. Carbon disulfide metabolites in the urine ranged from 0 to 950 mg/L. Cardiovascular effects, as assessed by blood pressure, blood coagulation, and measurement of serum creatine kinase activity, were not observed in 247 workers exposed to carbon disulfide at a median concentration of 4 ppm for a median duration of 4 years (Drexler et al. 1995b).

As described in Section 2.7, an interaction between carbon disulfide exposure and consumption of an atherogenic diet may lead to enhanced cardiotoxicity in rats. Rats administered carbon disulfide at 16 ppm and greater for up to 6 months exhibited concentration-related structural and functional changes (distention of the lumen, attenuation of myocardial vessels, irregular thickening of the aorta

wall, as well as microscopic histological changes). Although an increase in the enzyme activity (fructose-1,6-phosphatase, glutamate dehydrogenase, and glucose-6-phosphate dehydrogenase) was reported at the lowest concentration (3.2 ppm), the statistical significance of this finding was not reported. Also, no structural changes were seen at 3.2 ppm. However, when rats exposed to the same concentration of carbon disulfide were administered an atherogenic diet, there was an increase in mortality, a decrease in albumin and increase in globulin fractions in the serum, and serious metabolic and structural changes in the myocardium and the aorta (Antov et al. 1985).

Rats chronically administered 321.1 ppm carbon disulfide (5 hours a day, 6 days a week, for 15 months) did not develop any gross or histological lesions in the aorta; however, lipid droplets were occasionally noted on histological examination of the coronary arteries (Wronska-Nofer et al. 1980). In this same study, rats simultaneously fed an atherogenic diet had more advanced lipid infiltrates of the coronary arteries, which suggests that carbon disulfide may have an accelerating effect on atherosclerotic changes induced by dietary hypercholesterolemia. Thus, carbon disulfide may have promoted the development of atherosclerosis and coronary heart disease via altered cholesterol metabolism within the arterial wall.

Male Wistar rats exposed to 803 ppm for 18 hours showed reduced cardiac and respiratory rates and severe narcosis (Tarkowski and Sobczak 1971). However, this study used only six or seven animals and only one dose was tested.

Several studies have shown that carbon disulfide causes vascular changes in various organs of experimentally exposed animals. Acute inhalation (2 days) of 1,285 ppm in phenobarbitone-pretreated rats resulted in myocardial lesions characterized by necrosis, interstitial edema, and cellular infiltrate (Chandra et al. 1972). This effect was not observed in rats treated with carbon disulfide alone.

Gastrointestinal Effects. Nausea and vomiting were reported in approximately 50% of 123 persons following an accidental release of carbon disulfide in India (Kamat 1994). Gastrointestinal symptoms are also common among heavily exposed workers with carbon disulfide poisoning. In one study, 28% of the workers in a viscose rayon plant had a prevalence of symptoms (Vigliani 1954), and in another, viscose workers (n=l00) exposed to 1.9-26.4 ppm carbon disulfide in combination with 1.0-4.0 ppm hydrogen sulfide for 3 months to 17 years complained of stomach distress and impaired appetite (Rubin and Arieff 1945). Significant associations with nausea,

vomiting, and flatulence were also found in 119 carbon disulfide-exposed workers in a cross-sectional study by Vanhoorne et al. (1992b). Workers were exposed to l-36 ppm for an average of 4.2 years. These studies, however, are of limited value because of fluctuating exposure concentrations, concomitant exposure to other chemicals, and the fact that the reported symptoms are nonspecific and may have several etiologic agents.

No studies were located regarding gastrointestinal effects in animals after inhalation exposure to carbon disulfide.

Hematological Effects. Relative to 18 unexposed persons, fibrolytic activity was decreased in 57 workers exposed to carbon disulfide at 59-169 ppm for 2-8 years (Visconti et al. 1967). Serum plasmin concentrations decreased with increasing exposure duration. Red blood cell and white blood cell counts were not significantly different from preemployment values in 114 workers exposed to carbon disulfide at 0-21 ppm for 5 years (Chrostek-Maj and Czeczotko 1995a).

In animals, there is limited information that hematological effects occur following inhalation exposure. Brieger (1949) studied the bone marrow of four rabbits acutely exposed (6 hours/day for 12 days) to 1,100 ppm carbon disulfide and found no hematological effects. In the same study, longer exposure (6 hours/day for 48 days) to 300 ppm resulted in significantly increased pseudo-eosinophilic cells with a corresponding reduction in lymphocytes. It can be concluded that carbon disulfide increases the relative number of cells in the granulocytic series in the bone marrow. In another study, eight dogs exposed to 400 ppm carbon disulfide for 8 hours a day, 5 days a week, for 11 weeks did not exhibit adverse hematological effects or adverse alterations in blood chemistry except for a slight decrease in the serum albumin level after 2 weeks (Lewey et al. 1941). Significant depression in erythrocyte counts, total hemoglobin, and hematocrit were noted in mice exposed to 800 ppm, 6 hours daily, 5 days a week, for 90 days (Toxigenics 1983c). These studies suggest that hematologic effects are not consistent across species. Furthermore, these effects are likely to be dose- and duration-dependent. These changes do not correlate with clinical changes in the animals.

Hepatic Effects. Male volunteers exposed for 6 hours to graded concentrations (10-80 ppm) of carbon disulfide showed inhibition of oxidative demethylation of orally administered amidopyrine (Mack et al. 1974). In another study, Vanhoorne et al. (1992b) reported significantly increased liver size and γ-glutamyltransferase (GGT) activity in 119 carbon disulfide exposed workers compared to

79 controls. Workers had been exposed to l-36 ppm for a mean of 42 years. However, since adequate exposure data were not presented, this study should be interpreted with caution.

There is some evidence for increased serum cholesterol levels in workers exposed for prolonged periods to carbon disulfide levels in the range of 20-60 ppm, but most of these studies did not use an adequately matched control group (UK/HSE 1981). This study also measured total cholesterol levels and did not differentiate between high- and low-density cholesterol. Therefore, no interpretation is possible. An examination of total lipids, total and free cholesterol, and triglycerides in serum was conducted in workers with chronic exposure to carbon disulfide in a viscose rayon factory in Yugoslavia with time-weighted average (TWA) exposures of 4 ppm (n=58) or 18.5 ppm (n=102) over 17 years. Levels were compared to a nonexposed group of 41 workers (Krstev et al. 1992). Although there was a lack of dose response, i.e., total lipid and triglyceride levels were similar regardless of exposure, these levels were higher in exposed than in nonexposed workers. Again, no definitive conclusion can be established. In another study, workers chronically exposed to carbon disulfide in a viscose factory were classified by exposure duration of less than (n=17) or greater than (n=17) 20 years (El-Sobkey et al. 1979). Thirteen workers served as controls, and carbon disulfide concentrations ranged from 0.008 to 0.02 ppm for exposed workers. Exposed workers had significantly lower exposure duration-related mean values of thyroxine and higher mean values of free, esterified, and total cholesterol than controls. The study authors suggested that depression of serum thyroxine may be a key manifestation in hypercholesterolemia among carbon disulfide-exposed workers. The small sample size, lack of information on other exposures, and poorly characterized exposure concentrations preclude establishing conclusions from this study.

A significant positive trend for low-density lipoprotein cholesterol (LDL-Ch), total cholesterol, and diastolic blood pressure in workers exposed to carbon disulfide was observed in a cross-sectional study by Egeland et al. (1992). The Egeland study used existing data (Fajen et al. 1981) on 165 carbon disulfide-exposed workers and 245 unexposed controls recruited in 1979. The persons using medications to control ischemic heart disease and hypertension, as well as those using corticosteroid or thyroid medications, were excluded. Affected workers had been exposed for at least 1 year in a viscose rayon factory to a median 8-hour TWA of 7.6 ppm. The increases observed in total cholesterol were attributed to increases in LDL-Ch since there was no apparent effect on high-density lipoprotein cholesterol (HDL-Ch) ; triglyceride and fasting glucose levels were not associated with carbon disulfide exposure. Although these findings of an increased risk of arteriosclerotic heart

disease have been attributed to increased LDL-Ch, the correlation between carbon disulfide exposure and arteriosclerotic heart disease is not definitive in this study because of possible selection bias, cumulative exposure uncertainties, lack of control for coronary disease risk factors such as diet and exercise, and a limited statistical power to detect small changes in LDL-Ch.

Increased levels of LDL-Ch and apolipoprotein B as well as increased systolic and diastolic blood pressure indicative of increased coronary risk were noted in 115 carbon disulfide-exposed workers in a Belgian viscose rayon factory when compared to a control group of 76 workers (Vanhoorne et al. 1992a). Although these biochemical changes associated with cardiovascular disease were noted, no significant increases in prevalence of angina, myocardial infarction, or ischemia, as indicated by ECG changes, were found. In addition, elevated levels of apolipoprotein Al, which is a protective factor for coronary risk were also noted in exposed workers; this too may be related to the toxic effects of carbon disulfide exposure. Although the length of exposure was not specified, the authors noted that conditions in the plant had not changed since 1932 and that concentrations ranged from 1 to 36 ppm depending on job type. This study should be interpreted with caution since simultaneous exposure to low levels of H_2S also occurred. In addition, there may have been selection bias. Only 46% of those eligible for the referent group participated while referents with health complaints may not have participated, resulting in an underestimation of risk. Relative to preemployment values, increased triglycerides and β-lipoproteins were observed in workers exposed to 0-21 ppm carbon disulfide for 5 years (Chrostek-Maj and Czeczotko 1995a). A similar effect was not observed in 62 unexposed workers 5 years after a preemployment physical examination.

Compared to age-matched controls, an increase in total cholesterol, HDL-Ch, and LDL-Ch was observed in women 40-49 years of age and 50-59 years of age (Stanosz et al. 1994b). The women were exposed to carbon disulfide at 5-7 ppm for 0.5 to greater than 20 years. Only HDL cholesterol and LDL cholesterol were increased when the values were examined by duration of carbon disulfide exposure. Rather than being a hepatic effect, the investigators suggest that the effect may be on hormone production by the ovaries resulting in altered lipid metabolism.

No effects on serum cholesterol levels were noted in workers chronically exposed to l0-30 ppm carbon disulfide (Hernberg et al. 1971), and several studies failed to observe increased serum cholesterol levels in workers exposed to carbon disulfide at concentrations below 20 ppm. In an occupational study, 35 workers chronically exposed to carbon disulfide concentrations ranging from

6.4 to 12.8 ppm for 5-20 years exhibited a statistically significant reduction in blood cholesterol levels; a nonsignificant reduction in total lipid levels was also observed. This study is of limited value because of the small sample size and the likelihood of concurrent exposure to other chemicals (Sidorowicz et al. 1980). Another study of 70 men exposed to carbon disulfide in a viscose plant who were matched to unexposed men working in a different division of the plant found no statistically significant differences in blood lipid profiles (total cholesterol, HDL-Ch, and triglycerides) (Franco et al. 1982). Carbon disulfide concentrations were less than 11.2 ppm from 1972 to 1979. Workers (n=420) in a rayon filament factory chronically exposed to carbon disulfide (unspecified concentrations) for 4-25 years also exhibited no difference in total serum cholesterol, triglycerides, and β-lipoprotein in comparison to controls (n=390) (Sugimoto et al. 1978).

Only transient effects on liver metabolism have been observed in animals following inhalation exposure to carbon disulfide. Acute inhalation (8 hours) of 20 ppm carbon disulfide produced a reversible inhibition in oxidative drug metabolism by female rat liver microsomes and an increase in total hepatic lipid content (Freundt et al. 1974a). Rats exposed to much higher concentrations (642 ppm) of carbon disulfide for 4 hours exhibited no histological evidence of liver damage (Magos and Butler 1972; Magos et al. 1973). However, hepatotoxicity characterized by hydropic degeneration in parenchymal cells of the centrilobular zone was observed in rats pretreated with phenobarbitone to induce the liver mixed-function oxidase system and subsequently exposed to 642 ppm for 4 hours (Magos and Butler 1972; Magos et al. 1973). Starvation further potentiated the phenobarbitone-induced liver lesions in rats subsequently treated with carbon disulfide.

In mice, intermediate-duration inhalation exposures at a concentration of 482 ppm for up to 23 days (4 hours a day, 5 days a week) have resulted in a marked reduction in cytochrome P-450 and cytochrome c-reductase content after 2-3 days. The level returned to normal by the 23rd day of treatment. A significant decrease in uridine diphosphate-glucuronyl (UDP-glucuronyl) transferase was also noted, as well as a significant increase in lipid peroxidation (Jarvisalo et al. 1977a). Rabbits exposed to 300 ppm for 30 minutes a day for 120 days failed to develop any histopathologic alterations of the liver (Tsuyoshi 1959). However, because of the small sample size (n=3), the conclusions are preliminary.

The effect of carbon disulfide on lipid metabolism in the rat has been extensively studied (Wronska-Nofer 1972, 1973; Wronska-Nofer et al. 1978, 1980). Serum cholesterol, phospholipids, and

triglyceride levels were significantly elevated after exposure to 161-176.6 ppm carbon disulfide for 5 hours a day, 6 days a week, for 2 months or more (Wronska-Nofer 1972, 1973). A small but significant ($p<0.05$) increase was also noted in rats exposed to 74 ppm for 5 hours a day, 6 days a week, for 8 months (Wronska-Nofer 1973). Rats maintained on a Murigran chow *ad libitum* diet and exposed to 321 ppm carbon disulfide 5 hours a day, 6 days a week, for 6 months showed an increase in the rate of cholesterol influx from serum into the aorta wall. Those rats exposed under the same conditions for 8 months showed a slightly enhanced rate of aortic cholesterol synthesis (Wronska-Nofer and Parke 1978). Rats maintained on an atherogenic diet (2% cholesterol, 0.15% thiouracil) and exposed to 321 ppm carbon disulfide for 5 hours a day, 6 days a week, for 6 months had markedly increased serum and aortic cholesterol levels. These results suggest that carbon disulfide may increase arteriosclerotic changes resulting from diet-induced hypercholesterolemia (Wronska-Nofer et al. 1980).

Total serum cholesterol and fatty acids became elevated, but cholesterol esters decreased, in dogs administered 400 ppm carbon disulfide and simultaneously fed high-fat diets (Lewey et al. 1941). These data suggest that carbon disulfide, in conjunction with a high-fat diet, may lead to increases in cholesterol levels above those expected from high-fat diets alone. Rabbits (n=ll) exposed to higher concentrations of carbon disulfide (750 ppm) for 6 hours a day for 5 months exhibited a transient elevation in total serum cholesterol which was associated with weight loss (Cohen et al. 1959).

Thus, carbon disulfide does affect liver enzymes, particularly those related to lipid metabolism. The increases in serum cholesterol that are sometimes seen following carbon disulfide exposure may be a result of increased hepatic cholesterol synthesis.

Renal Effects. In a study with viscose rayon workers, there was a slight but statistically significant increase in the mean plasma creatinine concentration compared with the control group (Hernberg et al. 1971). However, the study authors concluded that all values were within reference ranges. Urinalysis did not reveal any effects on kidney function relative to preemployment values in 114 men exposed to carbon disulfide at 0-21 ppm for 5 years (Chrostek-Maj and Czeczotko 1995a).

Inhalation exposure of mice to 800 ppm for 6 hours a day, 5 days a week, for 90 days produced nephropathy (Toxigenics 1983c). Rabbits exposed to 300 ppm carbon disulfide for 30 minutes a day for 120 days failed to develop any histopathological alterations of the kidney (Tsuyoshi 1959). This study is of limited value because of the small sample size (n=3). An autopsy report of rabbits

(11 males) exposed to graded concentrations of 250-750 ppm carbon disulfide intermittently over a period of 38 weeks revealed an increased incidence of chronic interstitial nephritis (Cohen et al. 1959).

Endocrine Effects. The available human studies provide conflicting evidence on the adverse effects of carbon disulfide on thyroid function. However, these studies were limited by possible exposure to other chemicals, small sample size, and lack of quantification of precise exposure concentrations.

The effects of chronic exposure to carbon disulfide on serum thyroxine and cholesterol levels were studied in 50 workers employed in a viscose rayon factory (El-Sobkey et al. 1979). The control group consisted of 13 workers. The carbon disulfide concentration varied from 0.0083 to 0.02 ppm and the exposure duration from less than 20 years to greater than 20 years. An association was found between the lower serum thyroxine levels and serum concentrations of free and esterified cholesterol in exposed workers. According to the study authors, depression in serum thyroxine levels is related to metabolic disturbances leading to hypercholesterolemia among carbon disulfide-exposed workers.

In a study by Lancranjan et al. (1972), 109 workers exposed to carbon disulfide for 7-31 years were examined for thyroid function. A group of 40 workers served as controls. The exposure concentrations varied from 19 to 29 ppm and from 72 to 96 ppm. The study authors concluded that carbon disulfide did not induce thyroid alterations or disorders of lipid metabolism. In another study, the effect of long-term exposure to carbon disulfide (10-36 years) was studied in 15 exposed and 16 age-matched controls (Wagar et al. 1981). The exposure levels ranged from 10 to 51 ppm. No disturbance was noted in either thyroid function or serum prolactin values. Serum cortisol was also unchanged.

Urinary excretion of 17-hydroxycorticosteroids (formed from precursors of adrenal origin) and 17-ketosteroids (from both adrenal and gonadal sources) was reduced in workers exposed to carbon disulfide at 59-169 ppm for up to 8 years (Cavalleri et al. 1967). In a study that was designed to examine the effects of carbon disulfide on the sympathetic/adrenal system, decreased diurnal urinary excretion of adrenaline, decreased plasma dopamine, and increased serum β-hydroxylase activity were observed in women occupationally exposed at 5-7 ppm for periods ranging from 6 months to greater than 20 years (Stanosz et al. 1994a). Blood pressure was not significantly affected in this study, although among exposed women, a negative correlation was observed between daily urinary excretion of adrenaline and systolic blood pressure.

No studies were located regarding endocrine effects in animals after inhalation exposure to carbon disulfide.

Ocular Effects. Ophthalmological changes of various types, such as increased frequency of microaneurysms, related to the duration and intensity of exposure, have been found in Japanese workers. Severe ocular effects characterized by dot hemorrhages or microaneurysms of the retina were observed in Japanese workers exposed to carbon disulfide at levels between 5 and 15 ppm after 1955 and between 15 and 30 ppm earlier. The mean duration was 17 years in the first study (Sugimoto et al. 1978; Tolonen et al. 1976) and 10.8 years in the second study (Sugimoto et al. 1976) at concentrations averaging ≥20 ppm (high group) or <20 ppm (low group). Retinopathy, characterized by microaneurysms, was observed in 35% (43/124) of those exposed to air concentrations above 20 ppm carbon disulfide and in 23% (29/127) of those exposed to below 20 ppm, as compared to 4% (2/49) of the controls. These increases were statistically significant. The incidence and severity were shown to increase with longer durations of exposure to carbon disulfide. The retinopathy was not age related, the incidence being 37%, 29%, and 35% in exposed workers in the age groups 30-39, 40-49, and 50-59 years, respectively (Sugimoto et al. 1976). Although other exposures were not discussed, concurrent exposures to other chemicals may have also occurred.

A subsequent collaborative study was conducted to assess the incidence of retinopathy among groups of workers in Finland and Japan exposed to similar levels of carbon disulfide. The comparison showed that the differences in values obtained in the two studies were true differences and were not caused by interobserver variation (Sugimoto et al. 1977, 1978; Tolonen et al. 1976). Retinal red dots (microaneurysms and/or small hemorrhages) were observed in 25% (103/419) of the Japanese workers with chronic exposure to mean atmospheric levels of carbon disulfide of 15-35 ppm during the 1950s and below 20 ppm (5-15 ppm) since 1955, as compared to 4% (15/391) of the controls. However, no significant increase in the incidence of retinopathy was noted in Finnish workers exposed to 5-10 ppm carbon disulfide and to higher concentrations prior to 1970 (20-60 ppm during the 1950s and l0-30 ppm during the 1960s): 4% (7/188) were affected compared to 3% (2/76) of the controls. Thus, the prevalence of retinopathy among 419 Japanese workers exposed to 5-20 ppm carbon disulfide was high, whereas the incidence among 188 Finnish workers exposed to about 5-30 ppm was not greater than expected. The high prevalence among the Japanese workers may be explained by biased selection of exposed workers, different actual exposures, or the fact that no attempt was made in either study to account for the known risk factors for retinopathy (i.e., diabetes, hypertension). The

Finnish study may have selected for these findings. No data were presented regarding other possible exposures.

In studies of Finnish workers, 100 males with the longest and most marked history of exposure and 97 unexposed males were chosen for a neuro-ophthalmological investigation (Raitta and Tolonen 1975; Raitta et al. 1974, 1981). The 100 men had been exposed for between 1 and 27 years to 10-40 ppm; they were selected from the same cohort previously described by Hernberg et al. (1970). Corrected visual acuity, visual field, eye motility, pupillary reactions, and biomicroscopy were normal in all eyes examined. No retinopathy was detected in either group of individuals. However, delayed peripapillary filling of the choroid (both circumferential and segmental) occurred in 68 exposed and 38 unexposed eyes, a significant difference ($p \leq 0.01$). This was attributed to possible hemodynamic effects of carbon disulfide exposure. In addition, the mean widths of eight retinal vessels and the smallest vein were significantly greater in the exposed group, again attributed by the study authors to hemodynamic alterations with exposure. The study was limited by the difficulty in characterizing exposure levels because of the possibility that individual exposure varied widely and erratically over periods of time. However, a follow-up study validated the hypothesis that the delayed peripapillary filling of the choroid was related to the cardiovascular effects of carbon disulfide (Raitta and Tolonen 1975). In this follow-up, 38 male viscose rayon workers exposed to carbon disulfide were compared to 40 unexposed workers (previously examined neuro-ophthalmologically). Measurements were taken by oculosphygmography and were combined with individual electrocardiograms to statistically analyze the characteristics of the ocular pulse wave. Results showed that the exposed group of workers had a significantly lower pulse wave than that of the unexposed group, suggesting an increased rigidity of the ocular vascular bed in the viscose rayon workers. This study provided further evidence that carbon disulfide was not retinopathic in this Finnish cohort and in addition suggested a possible mechanism for the ocular effects of carbon disulfide.

To determine the effect of carbon disulfide exposure on retinal vasculature, American subjects from a viscose rayon plant (156 exposed, 233 unexposed) underwent pupillary dilation (with a short-acting mydriatic), direct ophthalmoscopy, and retinal photography with monochromatic light (NIOSH 1984a). Photographs were read by an ophthalmologist and rated as normal, as having definite or uncertain microaneurysms, or as having definite or uncertain hemorrhages. Subjects were categorized by job and characterized as having definitely low (DL<3 ppm), moderate (M=3-7.1 ppm), or definitely high exposure (DH>7.1 ppm). Retinal microaneurysms and hemorrhages were more prevalent in the

combined exposed groups than in the comparison group ($p \leq 0.04$). There was a concentration-related increase in the incidence of both definite and uncertain microaneurysms with exposure to carbon disulfide. No such trend was apparent for hemorrhages, nor for definite aneurysms alone. The combined exposed groups had almost 20% retinal microaneurysms (both definite and uncertain) compared to 7.5% for the comparison groups (significant, $p \leq 0.01$). The combined exposed groups had 10.5% retinal hemorrhages (both definite and uncertain) compared to 3% for the comparison group (significant, $p \leq 0.01$). The difficulties in characterizing dose levels and measuring the number of aneurysms by photography limit interpretation of the findings. Evidence regarding the occurrence of retinopathies due to carbon disulfide exposure is not uncomplicated. It appears that there may be a group of individuals, both Eastern and Western, who are genetically predisposed to respond with retinopathy to low levels of carbon disulfide exposure (NIOSH 1984a; Sugimoto et al. 1976, 1977). Conflicting evidence shows no retinopathy with exposure to slightly higher levels of carbon disulfide in the Finnish population (Hernberg et al. 1970; Raitta and Tolonen 1975; Raitta et al. 1974; Sugimoto 1977; Tolonen 1975; Tolonen et al. 1976). The differences in response by various populations have not been resolved.

Thirty workers in a viscose rayon plant were divided into two groups based on carbon disulfide exposure concentrations. The control group was exposed to average concentrations of 3.2 ppm, while the exposed workers experienced average levels of 16-32 ppm. Pigmentary changes and microvascular retinal lesions were observed in both groups (DeLaey et al. 1980; DeRouck et al. 1986). The study authors concluded that carbon disulfide affects several ocular structures and functions at low exposure levels. However, this study is limited by a small sample population, probable concomitant exposure to other chemicals, and an inappropriately chosen control group that was also exposed to carbon disulfide. In another study, viscose workers (n=l00) intermittently exposed to 1.9-26.4 ppm carbon disulfide in combination with 1.0-4.0 ppm hydrogen sulfide for 3 months to 17 years complained of burning of the eyes (Rubin and Arieff 1945). This study is of limited value because of fluctuating exposure concentrations, concomitant exposure to hydrogen sulfide and other chemicals, and lack of a control group.

Adverse ocular effects in workers of a viscose silk plant exposed 6 hours a day, 5 days a week, for 0.5-30 years to less than 3.2 ppm carbon disulfide were reported by Szymankova (1968). Disturbances were manifested as vascular or inflammatory degenerative changes in the retinas of 12

out of 75 (16%) of the exposed workers, which disappeared in 11 workers following cessation of carbon disulfide exposure.

Four female monkeys exposed to 256 ppm for 6 hours a day, 5 days a week, for 5-13 weeks suffered permanent visual impairment with degeneration of retinal ganglion cells compared to one control (Merigan et al. 1988). Visual acuity thresholds in two macaque female monkeys were severely disrupted after 5 weeks of intermittent exposure (6 hours a day, 5 days a week) to 256 ppm carbon disulfide. One monkey showed some recovery at 16 weeks postexposure; the other showed no improvement (Merigan et al. 1985). The observed effects were secondary to the effects on the optic nerve.

Body Weight Effects. Significant associations with anorexia were found in a cross-sectional study of 119 workers exposed to l-36 ppm carbon disulfide over a mean of 4.2 years (Vanhoorne et al. 1992b). This study, however, did not provide adequate exposure data. No information was located regarding effects on body weight in humans after inhalation exposure to carbon disulfide.

Female Wistar rats exposed to 800 ppm for 15 weeks (5 days a week, 6 hours a day) showed a 10% decrease in body weight gain (Hirata et al. 1992b). Male Long-Evans rats showed a 6-8% decrease at lower concentrations (350-600 ppm) following inhalation exposure for 10 weeks (Tepe and Zenick 1984). During a 14-day exposure (10 hours/day) to carbon disulfide at 600 ppm, male rats lost 14% of their body weight (Wilmarth et al. 1993). This concentration also resulted in a narcotic-like stupor in the exposed rats. Contrary to these findings, male Long-Evans rats that were exposed to 500 ppm for 5 or 12 weeks (5 days a week, 6 hours a day) showed no significant changes in body weight gain (Clerici and Fechter 1991). Female Long-Evans rats exposed to 800 ppm for 11 weeks (7 days a week, 7 hours a day) had a 15% decrease in body weight gain; this effect was not seen at 400 ppm (Rebert and Becker 1986). Inhalation exposure of Fischer 344 and Sprague-Dawley rats at 800 ppm for 90 days (5 days a week, 6 hours a day) caused a 16-30% decrease in body weight gain in both sexes (Toxigenics 1983a, 1983b). The percent of decrease in body weight gain depended on the strain of rat used (Toxigenics 1983a, 1983b). Inhalation exposure of Wistar rats to 546 ppm for 8 months or to 482 ppm for up to 14 months produced decreases in body weight (Szendikowski et al. 1974; Wronska-Nofer 1973).

Other Systemic Effects. Workers exposed for less than 5 years to TWA concentrations of 4.8-8 ppm had significantly elevated plasma sodium and chloride ions and decreased erythrocyte potassium and calcium (Pines 1982). However, the large variance in the electrolyte measurements among workers, the concomitant exposure to other chemicals, the fluctuating exposure concentrations, and the lack of a dose response for blood electrolyte alterations limit the value of this study.

Animal studies include a necropsy report on 10 male rabbits exposed to graded concentrations of 250-750 ppm carbon disulfide intermittently for 38 weeks that revealed increased adrenal weight, hyperplasia of adrenal cortex, and mild hemosiderosis of the spleen (Cohen et al. 1959). No information was given as to whether the controls underwent a sham exposure process, thereby controlling for the stress of the exposure procedure.

2.2.1.3 Immunological and Lymphoreticular Effects

The only study located that specifically addressed a possible immunological effect of carbon disulfide exposure in humans reported data that indicated that the β-lipoprotein isolated from carbon disulfide-exposed workers (presumably exposed via inhalation) is antigenically identical to lipoproteins isolated from healthy nonexposed controls (Bobnis et al. 1976). The authors concluded that these findings suggested no immunologic component involved in the increase of arteriosclerotic lesions found in carbon disulfide-exposed workers. There are no further data to either support or refute this conclusion.

No studies were located regarding immunological or lymphoreticular effects after carbon disulfide exposure in either humans or animals.

2.2.1.4 Neurological Effects

The primary target of carbon disulfide appears to be the nervous system. Neurophysiological and behavioral effects as well as pathomorphology of peripheral nervous system structures have been reported in humans as well as animals. Acute exposure to high concentrations of carbon disulfide can result in fainting and loss of consciousness. These effects were observed in 36-39% of 123 persons exposed to carbon disulfide following an accidental release of carbon disulfide, hydrogen sulfide, and sulfuric acid from a viscose rayon factory in India (Kamat 1994).

Most information available on neurotoxic effects of carbon disulfide in humans comes from occupational epidemiology studies. These exposures are considered to occur via inhalation, although some dermal exposures could have conceivably occurred, especially under conditions that may have prevailed 40-50 years ago. Separating possible effects of concomitant exposures to other chemicals can also present a problem. An examination of 118 male workers in a viscose rayon plant exposed for a median length of 15 years to carbon disulfide at an estimated average concentration of between 10 and 20 ppm revealed that the carbon disulfide-exposed workers had reduced maximal motor conduction velocity of the median, ulnar, deep peroneal, and posterior tibia1 nerves when compared to the controls (workers in a paper mill) (Seppalainen and Tolonen 1974). Individuals working in the plant before 1960 were exposed to higher levels (20-40 ppm) of carbon disulfide than were those working after this time. Furthermore, follow-up examination of these workers indicated that removal from the exposure environment did not lead to improvement of the nerve conduction velocity. However, it was noted that when individuals were removed from carbon disulfide exposure for l0-15 years, there was an equal division of people with either normal or decreased conduction velocities compared to a greater percentage of decreased velocities in individuals absent for 0-4 years. The authors of this study had earlier reported on neurophysiological findings in 36 workers exposed to high levels of carbon disulfide and described diminished nerve conduction velocities indicating polyneuropathy in many subjects (Seppalainen et al. 1972). Polyneuritis was reported to be present in almost all workers occupationally exposed to carbon disulfide for an average of 40 months at unspecified concentrations (Lancranjan et al. 1972). Overt polyneuropathy was reported in 9 of 17 male workers exposed to 150-300 ppm carbon disulfide for greater than 2 years, while 19 workers exposed to 15-150 ppm also had some symptoms of polyneuropathy (Chu et al. 1995). Nerve conduction velocities were significantly different in subjects with overt polyneuropathy when compared to subjects with subclinical effects and in subjects with subclinical effects when compared to unexposed controls.

Three groups of grain workers in three different work facilities (grain inspectors, malt laboratory workers, and grain elevator workers) showed various neurological effects. Their symptoms included distal sensory shading indicated by decreased sensitivity to pinprick and light touch, intention tremors, resting tremors, and nerve conduction abnormalities. The authors of this study concluded that the similarities of these symptoms to those reported in viscose rayon workers implicate carbon disulfide. The 21 subjects in this study were, however, self-selected, no controls were used, and no measurements of actual exposure to carbon disulfide were made (Peters et al. 1988). All individuals reported being able to smell the fumigant mixtures of carbon disulfide and carbon tetrachloride, and it

is suggested that at this point safe levels had been exceeded. Possible contributions to effects from the other components of the pesticide were not considered. It has been suggested that the symptoms of the grain workers exposed to the fumigant mixtures of carbon disulfide and carbon tetrachloride resemble those of patients with idiopathic Parkinson's disease (Matthews et al. 1990). Clinically, Peters et al. (1982) described loss of associated movements, cogwheeling, and atypical tremor in the grain workers resembling early Parkinsonism.

Clinical neurological examination of 16 men formerly exposed to carbon disulfide for at least 10 years revealed abnormalities in 15. Cerebral computerized tomography (CT-scans) showed signs of atrophy in 13, and neuropsychological examination indicated brain organic changes in 13. The authors of this study believed that long-term exposure to carbon disulfide involved a risk of developing toxic encephalopathy (Aaserud et al. 1988). Exposures were assumed to be between 9.6 and 19 ppm, with occasional higher exposures. There was no quantitation of individual exposures, however, and no adjustment was made to account for other possible occupational exposure or for lifestyle factors. CT-scans also revealed evidence of brain atrophy in 12 of 20 workers exposed to carbon disulfide at 0-21 ppm for 5 years (Chrostek-Maj and Czeczotko 1995b). The changes were observed most frequently in the frontal lobe. CT-scans were only completed in the 20 individuals with the worst psychiatric effects. Exactly what was being measured in the psychiatric examinations was not clear. In this study, psychiatric exams were completed before and 5 years after the start of exposure. In the exposed group of 114 men, the prevalence of "pseudoneurotic" symptoms increased from 8.4% to 43%. A similar increase was not observed among 62 unexposed control workers. Peters et al. (1988) noted that magnetic resonance imaging findings in 2 out of the 3 grain storage workers were indicative of central demyelination.

Regional cerebral blood flow was examined using Doppler ultrasound in 15 workers exposed to 3.2-28.9 ppm carbon disulfide for a mean of 20 years (Aaserud et al. 1992). Studies were performed 4 years after exposure ended. Asymmetrical blood flow patterns were observed in 8/14 workers, all of whom had encephalopathies consistent with carbon disulfide exposure. However, when the results were corrected to adjust for a possible influence of pCO_2, the values did not differ between the exposed workers and referents. No clear conclusions about this study can be made because of the small number of exposed workers, lack of a current exposure group, possible selection bias, and age variation between cohort and referents.

A study was conducted on 81 patients who had worked in environments containing toxic chemicals, who exhibited chronic carbon disulfide poisoning, and who showed evidence of polyneuropathy (Vasilescu 1976). The patients showed decreased conduction velocity in sensory nerves. These decreases were greater than those seen in the motor nerve velocities and, in some patients, occurred before any clinical signs. No measurements of actual exposure were given, however, and no estimates were made on the duration of occupational exposures or the chemicals that may have contributed to the total exposure history of the patients. Carbon disulfide was one of the major contaminants.

Workers exposed to carbon disulfide (n=145) were evaluated for its effects on the peripheral nervous system and compared to a group of nonexposed artificial fiber plant workers (n=212) located on the same premises (Johnson et al. 1983). The mean exposure period was 12.1 ± 6.9 years (mean ± SD), and individuals were divided into three groups based on previous exposure histories, job descriptions, and current carbon disulfide levels established on the basis of 8-hour personal monitors. The median carbon disulfide level for the comparison group was 0.2 ppm, while the median carbon disulfide levels of exposed individuals were 1, 4.1, and 7.6 ppm. The mean exposure concentration of all groups considered together was 7.3 ± 17.2 ppm (mean f SD) (23 mg/m^3), ranging from 0.6 to 16 ppm (1.9-50 mg/m^3). Carbon disulfide levels showed variability during breakdown periods at the plant which occurred “infrequently” but exposed some workers to brief periods of high carbon disulfide concentrations. Surface electrodes were used to measure maximum motor conduction velocity (MCV) in the ulnar and peroneal nerves and sensory nerve conduction velocity (SCV) in the sural nerve. There was a dose-related reduction in motor nerve conduction velocities in the calves and ankles, which was statistically significant in the high-concentration exposure group and in the average exposure group. However, the reductions were within the range of normal values. The study authors considered this to indicate minimal neurotoxicity. A chronic-duration MRL of 0.3 ppm was established for this effect, using 7.6 ppm as the LOAEL. In addition, reductions in the peroneal nerve conduction velocity appeared to be related to the workers’ cumulative exposure to carbon disulfide. This study reported health-related effects at average levels of exposure much less than those usually reported for occupational cohort studies. However, potential coexposure of these viscose rayon workers to hydrogen sulfide, tin oxide, zinc oxide and sulfate, sodium hydroxide, sulfuric acid, and lead may account for a portion of the toxicity response.

Mental performance and personality disorders were examined in 17 long-term workers from each of two rayon factories, one group with a long history of relatively high exposures to carbon disulfide

(mean concentration of carbon disulfide measured after 1971 was 57.8 ppm) and the other with lower exposures (mean concentration of carbon disulfide measured after 1971 was 19.3 ppm). Twenty-one test variables were used: 3 intelligence tests, 6 personality tests, and 12 ability measurements. The workers from the factory with the highest exposure measurements showed more anxiety, introversion, and depression than the other workers. They also did significantly worse on tests designed to measure number facility, sustained attention, speediness, and carefulness. There were, however, no estimates of individual exposure and no control for concomitant exposure to other chemicals (Foa et al. 1976). Lack of attention and reduced perceptive ability were observed in viscose rayon workers exposed to carbon disulfide at 0.6-2.6 ppm with peaks of 11.2 ppm (Cassitto et al. 1993). The investigators suggest the effects may be a result of transient peak concentrations of carbon disulfide rather than the low concentrations. Other studies have indicated that grain storage workers had intense exposures interspersed often with periods of more minimal contact with anti-weevil chemicals (Matthews et al. 1990; Peters et al. 1982, 1986a, 1986b, 1988).

Behavioral examinations (psychological tests, psychomotor tests, and cognitive-perceptual tests) of 131 workers in a rayon plant who were exposed to carbon disulfide were compared to those of 167 workers who worked in textile plants that manufactured other synthetic fibers. Exposure and companion (control) groups and exposure levels are the same as those described for the Johnson et al. (1983) study. The workers completed a checklist of symptoms characteristic of various neurobehavioral syndromes. The results showed no behavioral changes of any major significance. The rayon workers did report symptoms of neurobehavioral ailments, however. Workers were classified individually according to job title and the past and present exposure levels for individuals in that job title. The exposures measured in the plant were generally below 20 ppm, suggesting that these levels may be too low to identify behavioral changes (Putz-Anderson et al. 1983).

A study of neuropsychological variables in carbon disulfide-exposed workers investigated 120 workers selected on the basis of age not exceeding 50 years and an absence of family or personal history of nervous disorders. The test battery consisted of three intelligence tests, three personality questionnaires, a test of memory involving measures of perception, recognition, and free recall, and two performance measures. Workers were grouped according to exposure categories (none; low, about 20 ppm; medium, between 20 and 38 ppm; and high, greater than 38 ppm). The no-exposure and low-exposure categories were combined for analysis. Differences in the groups' measurements were statistically analyzed and revealed decreased intelligence scores, performance, and memory and

increased fatigue and depression in the workers with higher exposures. These changes were dose related, although the exposure variable was categorical and not quantitative (Cassitto et al. 1978; Cirla et al. 1972). Neurological effects in 50 viscose rayon workers occupationally exposed to an average concentration ranging from 3.2 to 9.6 ppm carbon disulfide for 3-12 years were compared to a pair-matched control group from unexposed departments of the plant (Cirla and Graziano 1981). There were no significant differences in the exposed and control groups for three considered parameters: maximum and minimum conduction velocity and residual latency on the peroneal nerve. Psychological examinations of 25 pairs showed no apparent differences between the exposed and control groups. Electromyography and clinical diagnosis revealed only one exposed worker with minimal neuropathy. Furthermore, four exposed and two unexposed workers were clinically diagnosed with the nontoxic syndrome and radiculopathy, and no cases of polyneuropathy were found in either group. Clinical diagnosis of central impairment was found in two exposed workers and no unexposed workers with psychoorganic syndrome. The number of neuropathy or psychoorganic cases was not significant enough to associate exposure to carbon disulfide with adverse neurological effects. Furthermore, the absence of significant differences in peripheral nerve motor conduction velocity and psychological parameters lends particular weight to the conclusion that occupational exposure below 9.6 ppm (maximum exposure concentration) for up to 12 years does not cause recognizable adverse neurological health effects.

In a cross-sectional study of the chronic effect of carbon disulfide exposure on the central nervous system, researchers measured the brain stem auditory evoked potential (BAEP) in Japanese spinning workers from a viscose rayon factory (Hirata et al. 1992a). The workers were divided into three groups depending upon length of exposure: 34 current workers exposed for more than 240 months, 24 current workers exposed for 24-84 months, and 16 former workers exposed for more than 120 months. The 39 unexposed controls were workers in a nylon filament factory. The TWA exposures ranged from 3.3 to 8.2 ppm (mean 4.76 ppm). The latencies of the three main components of BAEP increased compared to those in the control group. The significantly higher interpeak latencies in workers exposed to carbon disulfide for more than 240 months suggest that chronic exposure to carbon disulfide involves the auditory ascending tract in the brain stem. Despite long exposure, BAEP parameters in workers exposed to more than 120 months were not significantly higher than those of the control group.

Neuropsychological and neuropathological examinations were performed on 16 viscose rayon workers in Norway exposed to 3.2-28.9 ppm for an average of 20 years (Aaserud et al. 1990). Workers were also exposed to hydrogen sulfide (80 mg/m^3) while working. The clinical tests performed included electroencephalograms (EEG), electromyograms (EMG), computerized axial tomography (CAT) scans, and motor and sensory neurography. Workers complained of dyspnea, tiredness, nausea, decreased memory, and irritability. Major neurological deficits occurred in 6/16 workers, while minor deficits occurred in 9/16. Pathological changes in EEG patterns were noted in two workers while six showed pathological changes in EMG. Decreased nerve impulse conduction and motor, sensory, and mixed motor/sensory neuropathies were demonstrated. Cerebral or cerebellar atrophy was noted in 13/16 workers, and neurophysiological examinations showed psychomotor retardation and coordination difficulties. Study deficiencies were the lack of a control group and confounding factors in the subjects, including alcohol abuse.

The neurotoxic effects after 10 years or more of long-term, low-level occupational exposure to carbon disulfide in workers at a viscose rayon plant were examined by assessing markers of the peripheral and autonomic nervous system (Ruijten et al. 1990, 1993). Reinvestigation of 44 of 45 exposed and 31 of 37 matched control workers revealed changes in the motor nerve conduction velocity (Ruijten et al. 1993). The exposure concentration in the two studies varied from 1 to 30 ppm. For peripheral nerves, a decrease in the conduction velocity in both fast and slow motor nerve fibers (peroneal nerve) was observed in exposed workers. Sensory conduction velocities were reduced and the refractory period of the sural nerve was increased. The effects on the sural nerve were pronounced. A small decrease in conduction velocities in the absence of symptoms of neuropathy and decreased response amplitudes suggest a mild presymptomatic nerve impairment.

The relationship between electric impulse transmission and visual stimuli was examined in a group of 21 patients with chronic carbon disulfide exposure in a rayon production plant for 20-36 years and control groups of 25 or 36 healthy unexposed males (Sikora et al. 1990). A significant correlation was observed in latency and amplitude of response. The correlations suggest cerebral dysfunction of the visual pathway and diminished ability to transform visual information to motor reaction at the level of the cortical association center. The study was limited by the lack of quantification of exposure levels and the variability in responses in the exposed group.

Finger tremor accompanying voluntary movement was studied in 19 control subjects and 19 grain workers exposed to carbon disulfide-based fumigants for 13.5 years (Chapman et al. 1991). Comparison was made between finger tremor detected using computerized techniques and Parkinsonian tremor detected visually on neurological examination. The measurement of amplitude and frequency provided a more accurate diagnosis than the visual observation. The distribution of tremor frequency power in the grain workers was reminiscent of tremor in idiopathic Parkinson's disease. These findings suggest that the measurement of subtle tremor frequency changes may provide an early indication of chronic carbon disulfide poisoning. However, the study was limited by the lack of exposure concentrations and the use of only symptomatic cases.

Animal studies on the neurotoxicity of carbon disulfide have usually been done in rats and provide histopathologic and neurochemical data that support a neurotoxic effect for carbon disulfide. In general, the doses used in these animal studies are considerably higher than the occupational exposures seen in epidemiological studies.

In a study that examined effects in rats (Wistar) and mice (H strain) exposed to carbon disulfide for 4 hours, rats appeared to be more sensitive than mice (Frantik et al. 1994). The concentration that resulted in a 30% inhibition of electrically evoked seizure discharge was 1,370 ppm in male rats and 2,600 ppm in female mice.

Short-term exposures to inhaled carbon disulfide in rats have shown consistent results with respect to brain chemistry changes and sensory and motor nerve conduction alterations. Rats exposed for 4 hours a day for 10 days at 642 ppm showed decreased noradrenaline, increased dopamine, and elevated tyrosine in the brain (Magos and Jarvis 1970). The authors proposed that changes in tyrosine, dopamine, and noradrenalin may be caused by a feedback mechanism in which the increase in dopamine prevents a conversion of tyrosine to dopamine. No relationship to clinical signs or behavioral effects was noted. Further work at the same exposure level indicates that the noradrenaline concentration remains significantly decreased for at least 20 hours after a l-hour exposure, but the dopamine levels return to normal (Magos et al. 1974). Female rats exposed to 777.1 ppm for 12 hours showed swollen brain mitochondria and elevated brain adenosine triphosphate (ATP) levels compared to controls (Tarkowski et al. 1980). However, no histopathological changes were noted in brain tissue. Under the same experimental conditions, rats exposed to 257 ppm for 5 hours a day, 5 days a week,

for 10 months showed biochemical changes that involved uncoupling of oxidative phosphorylation (Tarkowski et al. 1980).

Mice exposed to 0, 120, 580, 2,200, or 3,700 ppm carbon disulfide for 1 hour showed no behavioral changes at 120 ppm but did at 580 ppm and higher (Liang et al. 1983). The same study investigated an intermediate-duration exposure as well. Mice were exposed to 0, 260, 580, or 840 ppm carbon disulfide for 4 hours a day, 5 days a week, for approximately 30 days. No changes were observed at 260 ppm, but concentration-related decreased responses to operant behavior were observed at 580 ppm and at 840 ppm. The study is limited because there is no indication of the number of animals used. For the 30-day exposure study, there was no truly unexposed control, since some of the mice from the acute phase were used. The same mice were used for all doses in the intermediate phase, with l0-14 days between each dose exposure. It is therefore unclear whether the effects noted were due to the dose or were a cumulative effect of previous doses.

Male Wistar rats were exposed to 578 ppm carbon disulfide for 10 months. Also reported in the same paper were results from an 18-hour exposure to 803 ppm. The rats developed different signs of poisoning depending upon the type of exposure: the l0-month exposure caused loss of motor equilibrium, muscular weakness, and hind-limb paresis, and the acute dosing caused severe narcosis, reduced cardiac and respiratory rate, straightening of hind limbs, and lower body temperature. However, brain mitochondria in both groups of animals exhibited the same types of disturbances in oxidative phosphorylation-uncoupling of oxidative phosphorylation, decreased phosphorus-oxygen (P:O) ratio, and a lower ATP-inorganic phosphorus (ATP-P_i) exchange rate (Tarkowski and Sobczak 1971). Although this study used only six or seven animals and one dose, the results were consistent and do not conflict with other information regarding the effects of carbon disulfide on metabolism.

A narcotic-like stupor was observed during carbon disulfide exposure of rats at 600 ppm 10 hours a day for 14 days (Wilmarth et al. 1993). By the end of the study, mild ataxia and moderate hind-limb splay were also observed. Neurobehavioral effects were observed in rats exposed to 642.2 ppm of carbon disulfide for 4 hours a day, 5 days a week, for 6 weeks. No changes were seen after 3 weeks of exposure, but hind-limb extensor responses and motor coordination were impaired after 6 weeks of exposure. Recovery had occurred by 3 weeks after cessation of exposure. The carbon disulfide-exposed rats were stimulated less than the air-ventilated controls by 3 mg/kg of *d*-amphetamine, suggesting that repeated exposure to carbon disulfide affects the availability of brain

noradrenaline for release. This effect also disappeared by 3 weeks after exposure (Tilson et al. 1979). These results are consistent with those of Magos and Jarvis (1970) and Magos et al. (1974) (discussed above) in which noradrenaline was shown to be decreased in the brains of acutely exposed rats.

Motor capacity (static endurance and dynamic performance at forced motor activity) was studied in a total of 96 albino rats that were repeatedly exposed to 0 (42 males/group), 48, 385, or 770 ppm carbon disulfide via inhalation (18 males/group) (Frantik 1970). Acute toxicity was measured 0-60 minutes after termination of exposure, and chronic toxicity was measured 48-72 hours postexposure. After initial exposure to the 770-ppm dose there were reductions in spontaneous motor activity (60%), conditioned avoidance, and motor performance. Effects persisted for 24 hours but disappeared completely 3 days postexposure and failed to reappear after repeated experiments. Symptoms of motor impairment were observed after a variable latent period and were related to exposure concentration (385-ppm dose, 18 weeks; 770-ppm dose, 8 weeks). On the average, motor capacity (maximum speed and endurance at dynamic performance) was reduced by 40-50% at the 385-ppm dose and by more than 80% at the 770-ppm dose. Motor function recovered during the first 8 weeks. The study is limited by the lack of quantitative measurement of nervous system impairment.

Neuromuscular and sensory effects were evaluated in Long Evans rats exposed to 500 ppm (6 hours a day for 5 or 12 weeks) using an acoustic startle test (Clerici and Fechter 1991). Neuromuscular integrity was shown to be compromised based on auditory startle reflex amplitude; animals showed a 70% recovery 4 weeks postexposure. No clinical signs of neurotoxicity or changes in hearing function or acoustic tone thresholds were noted. Use of a single exposure concentration precluded assessment of dose response.

The chronic effect of carbon disulfide exposure on the central nervous system was examined by auditory brainstem responses (ABR) in female JCl Wistar rats (Hirata et al. 1992b). Rats were exposed by inhalation to 200 or 800 ppm, 6 hours a day, 5 days a week, for 15 weeks. Auditory responses were measured before exposure, every 3 weeks during exposure, and in weeks 2 and 6 after exposure. The delayed latencies of ABR were observed at 800 ppm suggesting a conduction dysfunction. The transient delay of ABR responses at 200 ppm indicated only slight conduction dysfunction. Rats recovered 2-6 weeks after carbon disulfide exposure.

Neuropathology has been investigated in several studies of carbon disulfide exposure in rats. The results are consistent with regard to effect but not with regard to the dose required to produce the effect. Axonal swellings, demyelination at axonal enlargements, swelling of nerve terminals at neuromuscular junctions, muscle atrophy and degeneration, damage of the terminal axons, myelin indentation, fiber breakdown, and distended mitochondria have been reported (Jirmanova and Lukas 1984; Juntenen et al. 1977; Szendzikowski et al. 1974). These experiments used only one dose, which varied between 482 and 770.7 ppm, and a control group; therefore, no dose response can be established using only these data. A study by Rebert and Becker (1986) attempted to establish a temporal dose-response relationship for peripheral nerve conduction activity. Their work with rats showed that visual-evoked potentials and conduction time in peripheral nerves and in brainstem auditory pathways were longer in animals exposed to 800 ppm, 7 hours a day, 7 days a week than in those exposed to 400 ppm for the same duration (11 weeks). The potentials in the groups exposed to lower levels were longer than in the controls, although the differences were not statistically significant. Four female monkeys exposed to 256 ppm for 6 hours a day, 5 days a week, for 5-13 weeks suffered permanent visual impairment with degeneration of retinal ganglion cells (Merigan et al. 1988). Visual acuity thresholds in two macaque female monkeys were severely disrupted after 5 weeks of intermittent exposure (6 hours a day, 5 days a week) to 256 ppm carbon disulfide. One monkey showed some recovery at 16 weeks postexposure; the other showed no improvement (Merigan et al. 1985).

Neurological effects such as hind-limb motor difficulties, reduced nerve conduction velocity, and degeneration of nerve fibers were seen in rats exposed to 700 ppm of carbon disulfide for 5 hours a day, 5 days a week, for 12 weeks (Colombi et al. 1981). These pathologies continued to 3 weeks postexposure but were slightly improved 6 weeks after carbon disulfide exposure. The improvement continued up to the 18th week of recovery, suggesting that the process may be reversible. This study was limited by the use of a single carbon disulfide dose. In another study, paralysis of hind limbs was observed in Wistar rats exposed to 546 ppm for 8 months (5 hours a day, 6 days a week); this effect was not seen at 321 ppm (Wronska-Nofer 1973).

Morphological changes in the peripheral nerve and the spinal cord were studied in rats and mice exposed to 50, 300, or 800 ppm carbon disulfide, 6 hours a day, 5 days a week, for 90 days (Gottfried et al. 1985; Toxigenics 1983a, 1983b, 1983c). Rats exposed to 50 ppm showed no changes in any parameters; rats exposed to 300 ppm showed only occasional swelling of axons in dorsal corticospinal

fibers of the lumbar spinal cord; and rats exposed to 800 ppm showed extensive neurofilamentous axonal swelling in the spinal cord. Neurofilamentous axonal swelling was particularly seen in the distal portion of long fiber tracts, including prominent swellings in the dorsal ascending sensory fibers, whereas it was only intermittently seen in the dorsal corticospinal fibers. In addition, extensive peripheral nerve changes were seen at the level of the posterior tibia1 nerve. The sciatic nerve showed no appreciable loss and only occasional axonal swelling. Ultrastructurally, the axonal swellings contained abundant disorganized neurofilaments, decreased microtubules, and thin or absent myelin. Brain and body weight were decreased in proportion to the concentration, with the decrease in brain weight statistically significant in the 800-ppm group.

The highest NOAEL values and all reliable LOAEL values for neurological effects are recorded in Table 2-1 and plotted in Figure 2-l.

2.2.1.5 Reproductive Effects

Data on reproductive effects of carbon disulfide in humans come from studies of occupational cohorts that are exposed primarily via inhalation to carbon disulfide in the workplace. These studies are limited by generally poor exposure measurements, concomitant exposures to other chemicals, and occasionally the lack of appropriate control groups. Nonetheless, the data provide some evidence that carbon disulfide may act on the reproductive system.

In some studies, effects in females included an increased incidence of spontaneous abortion at levels as low as about 2 ppm (6-7 mg/m^3) (Heinrichs 1983; Wang and Zhao 1987). Other epidemiological studies have not corroborated these reports, however. In a community study of spontaneous abortion, occupation, and air pollution, the study authors found no relationship between carbon disulfide concentrations and miscarriage rates (Hemminki and Niemi 1982). Another study reported that women exposed to 0.5-4.7 ppm (1.7-14.8 mg/m^3) had significantly more menstrual disorders than nonexposed women; however, there was no increase in the rate of spontaneous abortion, stillbirth, premature delivery, or congenital malformation (Zhou et al. 1988). Increased rates of menstrual disorders and toxemia of pregnancy were also reported in workers exposed to 12-18 ppm carbon disulfide (Cai and Bao 1981). However, concomitant exposures to other chemicals were not considered.

Researchers examined 15 men exposed in a viscose plant to unspecified concentrations of carbon disulfide and hydrogen sulfide for 10-36 years and compared them with 16 age-matched controls (Wagar et al. 1981). The carbon disulfide concentrations at the viscose plant were below 10 ppm just prior to the study, but the levels had been higher previously. Serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were significantly increased in the exposed workers. The study authors concluded that this was a sign of primary gonadal insufficiency. However, no changes were seen in serum testosterone or thyroid functions. Exposures were not well characterized, only a small number of workers were examined, and semen analysis was not performed; these limitations preclude developing conclusions from this study. In a National Institute for Occupational Safety and Health (NIOSH) study of 434 American workers, semen quality was evaluated in 86 exposed and 89 unexposed workers (Meyer 1981). The duration of carbon disulfide exposure ranged from 12 months to 257 months. Statistical analysis of sperm count, ejaculation volume, and morphology patterns showed no statistically significant difference between the exposed and unexposed groups. Exposures were not well characterized and were designated as definitely high (DH=18 workers), moderate (M=27 workers), definitely low (DL=22 workers), and difficult to quantify or other (O=19 workers). A further limitation was the possibility that the poor rate of worker participation (50%) in the semen sampling could have affected the results. In another workplace study, men (n=116) exposed to carbon disulfide at 0.3-9.6 ppm or >9.6 ppm complained of decreased libido and impotence more frequently than 79 unexposed controls (Vanhoorne et al. 1994). Reproductive histories did not reveal any effects on fertility. Examination of semen from 43 exposed workers and 35 controls did not show any adverse effects.

A review of some reports from the USSR and other Eastern Bloc countries indicates that, although these studies may be deficient in design and reporting of data, there is enough evidence to warrant concern about the effects of carbon disulfide on the female reproductive system. Reported adverse effects included menstrual disturbances, endocrine alterations, and increased incidence of miscarriage (Zielhuis et al. 1984).

Effects in males occupationally exposed to carbon disulfide have included teratospermia, decreased sperm motility, hypospermia, and decreased libido (Lancranjan 1972). Studies have stressed the importance of assessing the statistical power of a test for comparing sperm count or morphology (Wyrobek 1983) and the need for more clearly defining the link between changes in morphology and altered fertility (Schrag and Dixon 1985). The relationship between paternal exposure to carbon

disulfide and pregnancy was studied in 540 workers and their wives (NIOSH 1983). Patterns of fetal loss, number of births, and time between live births were examined by comparing pregnancies conceived after paternal exposure to carbon disulfide to pregnancies occurring in an unexposed population. Several statistical analyses showed no relationship between carbon disulfide exposure and fertility measures.

Evidence in animals supports the effect of carbon disulfide on the reproductive system. Male Long-Evans rats exposed to 600 ppm carbon disulfide 5 or 6 hours a day, 5 days a week, for 10 weeks showed significant alterations in copulatory behavior and a decrease in ejaculated sperm counts by the 4th and 7th weeks of exposure, respectively. Caudal epididymal sperm counts were not depressed and testes appeared histologically normal. The plasma testosterone levels were significantly reduced. Exposed rats had significantly reduced weight gains during the experiment (Tepe and Zenick 1984; Zenick et al. 1984).

The NOAEL and LOAEL values for reproductive effects in animals are recorded in Table 2-l and plotted in Figure 2- 1.

2.2.1.6 Developmental Effects

Developmental effects of carbon disulfide have been reported in several animal studies. Inhalation exposure of pregnant rats did not produce congenital malformations at levels of 3.2 and 0.01 ppm. However, administration of 3.2 ppm was also associated with viability impairments and retardation of morphological and sensory development, and 0.1 ppm was associated with behavioral changes (Tabacova and Balabaeva 1980b). To study the reproductive toxicity and teratogenicity of carbon disulfide, other inhalation exposure studies of pregnant Wistar or Sprague-Dawley rats and New Zealand white rabbits exposed to 20 or 40 ppm carbon disulfide were conducted (Hardin et al. 1981; NIOSH 1980). Results showed an absence of maternal toxicity, fetal toxicity, and teratogenicity. These studies were limited by the absence of information regarding exposure conditions and the presentation of results as a summary of 10 tested chemicals with little experimental detail.

A developmental study was conducted using New Zealand White rabbits, which are more sensitive than rats to the effects of carbon disulfide (PA1 1991). In this study, rabbits (24/group) were exposed by inhalation to 0, 60, 100, 300, 600, or 1,200 ppm, 6 hours a day, on gestation days 6-18. Animals

were evaluated on day 29. Maternal toxicity was observed as reduced body weight gain and adverse clinical signs (ataxia, lowered food consumption, wheezing) in the 1,200-ppm group, with some sporadic hematologic alterations at 600 ppm (for example, decreased hematocrit on gestation day 19). These effects were not seen in an initial range-finding study in which rabbits were exposed to 1,000 ppm carbon disulfide. Embryotoxic effects (reduced mean fetal body weight) and post-implantation loss were seen in the 600- and 1,200-ppm exposure groups. Teratogenic effects (increased cumulative skeletal and visceral malformations) were also seen at 1,200 ppm carbon disulfide. In this study the NOAEL for developmental effects was 300 ppm and the NOAEL for maternal toxicity was 600 ppm because of the lack of biological significance in the hematologic findings at this exposure concentration.

In another developmental study, B6C3Fl mice and Fischer and Sprague-Dawley rats were exposed to 0, 49, 297, or 798 ppm carbon disulfide 6 hours a day, 5 days a week, for 90 days (Toxigenics 1983a, 1983b, 1983c). Examination of the mice and rats that received the highest dose revealed maternal toxicity but no developmental toxicity.

Based on data from mice exposed for 10 minutes to 750 ppm ^{14}C-labelled carbon disulfide, carbon disulfide and its metabolites pass the placenta at all stages of gestation and localize selectively in tissues reported to be target organs for the effects of this chemical (brain, blood, liver, and eye) (Danielsson et al. 1984). This finding provides evidence that carbon disulfide or its metabolites may exert effects directly on the embryo.

Maternal reproduction and fetal parameters were evaluated for groups of 20-23 pregnant Sprague-Dawley rats exposed to 0, 100, 200, 400, and 800 ppm carbon disulfide, 6 hours a day, during days 6-20 of gestation (Saillenfait et al. 1989). Maternal toxicity for the animals exposed to 400 or 800 ppm was evidenced by the significant decrease in maternal body weight gain (19% and 31% decrease) of the exposed rats compared to the controls ($p \leq 0.01$). When gravid uterine weight was subtracted from the dam's body weight gain, the maternal weight was significantly reduced by 56% and 144% versus the controls. Exposure to 400 or 800 ppm carbon disulfide resulted in a significant reduction of fetal body weights for both sexes; the respective weight reduction from control for males was 7% and 14% ($p \leq 0.0l$), and for females was 6% and 20% ($p \leq 0.01$). Clubfoot was the only external malformation occurring at higher frequency than in the controls; however, the one fetus affected in one litter at 400 ppm and the seven fetuses affected in five litters at 800 ppm were not

sufficient for statistical significance. Incidence of unossified stemebrae was significantly increased in groups exposed to 800 ppm ($p \geq 0.0l$), but no other soft tissue anomalies or major skeletal anomalies were present in any treated groups.

Behavioral and neurotoxic effects in the offspring of rats exposed via inhalation have been reported. Perinatal mortality was shown to be dose related to prenatal carbon disulfide exposure levels (225 and 642 ppm) in rats (Lehotzky et al. 1985). Exposure to 642 ppm carbon disulfide throughout pregnancy for 2 hours daily produced no malformations of fetuses in rats or mice but did increase the death rates of the embryos at all stages of intrauterine development (Yaroslavski 1969).

In a developmental study in which only the dams of both F_0 and F_1 generations were dosed during gestation, the F_1 offspring of albino rats were exposed for 8 hours daily throughout pregnancy to 64.2, 32.1, 3.2, or 0.01 ppm carbon disulfide and were postnatally exposed to the same exposure concentrations for the same duration (Tabacova et al. 1983). Both the F_1 and F_2 generations showed a marked increase in malformations and behavioral and learning changes at the highest concentration, but the F_2 generation also showed the same effects at the two lower concentrations. Maternal toxicity and teratogenic effects were observed in offspring of dams of all generations exposed to 32 or 64 ppm carbon disulfide. The two lower exposure levels, although nonteratogenic, provoked functional and behavioral disturbances of varying degree. The study authors suggested that carbon disulfide causes preconditioning to increased effects in the next generation. There are, however, no other data that support a conditioning effect of carbon disulfide. This study is limited by the lack of information on chemical exposure (including chemical purity, atmosphere verification, or analytical techniques) on the control animals, and on the methods used to select F_1 and F_2 generations. In addition, there was in excess of a 300-fold difference in concentration below the lowest treatment level (0.01 ppm) and the next higher level (3.2 ppm); there was not a clear dose response; there was no information on mode of control exposure; there was a lack of concurrent controls (separate control animals were used for higher and lower exposure animals); animal diet and housing conditions were not specified; and there was a lack of information regarding the significance of the increased behavioral effects measured in exposed animals (reduced exploratory activity and “increased emotional activity”). Finally, the low effect levels found in this study were not substantiated in subsequent inhalation studies by Saillenfait et al. (1989) and PAI (1991). However, the design of the Tabacova et al. (1983) study was significantly different from both the Saillenfait et al. (1989) and PAI (1991) studies. The lack of

substantiation of the Tabacova et al. (1983) study may be due to differences in the designs of the Saillenfait et al. (1989) and PAI (1991) studies.

All reliable LOAEL values for developmental effects are recorded in Table 2-l and plotted in Figure 2-1.

2.2.1.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans after inhalation exposure to carbon disulfide.

Carbon disulfide failed to produce significant chromosomal aberrations in the bone marrow of rats inhaling 20 and 40 ppm for either acute or intermediate periods (NIOSH 1980).

Other genotoxicity studies are discussed in Section 2.5.

2.2.1.8 Cancer

There is no definitive evidence for an increased cancer potential from carbon disulfide in humans. The number of deaths due to neoplasms was compared in a cohort of rayon plant workers versus paper mill workers, and no significant differences in mortality were found between the years 1967 and 1982 (Nurminen and Hernberg 1985). Thus, there appears to be no association between occupational exposure to carbon disulfide and cancer in this study. (This information was reported incidentally in another study examining cardiovascular mortality, described in Section 2.2.1.2.) On the other hand, data supporting an increased odds ratio for lymphocytic leukemia in rubber workers exposed to several different kinds of solvents including carbon disulfide have been reported (Arp et al. 1983). Categories of exposure were based on process descriptions for the person's job classification and not on ambient air measurements. Jobs incorporating benzene use were specifically excluded. The odds ratio was not, however, statistically significant at $p<0.05$. Data did indicate a statistically significant ($p<0.00l$) odds ratio for exposure to carbon disulfide and development of lymphocytic leukemia when researchers carefully analyzed specific exposures in the group to solvents other than benzene (carbon disulfide, toluene, xylene, naphtha, ethanol, acetone, hexane, phenol, trichloroethylene, trichloroethane, and 13 others). This odds ratio was higher than that detected for benzene, which was not statistically

significant (Checkoway et al. 1984). Deaths from lymphatic leukemia in this same cohort were also shown to be associated with exposure to carbon disulfide (Wilcosky et al. 1984). There are several potential confounding factors in these analyses. All three reports are based on nested case-control studies from the same cohort of rubber workers. The small number of cases for each particular type of cancer examined and the large number of solvents used in the analysis indicate a need for cautious interpretation. Many of the solvents were used in mixtures so that identifying a single causal agent is not possible. Confounding factors from nonoccupational or other occupational exposures were not taken into account. In addition, the system designed to estimate historical exposures may have had weaknesses, specifically that the designation of “permitted to use” may not indicate actual use.

No studies were located regarding cancer in animals after inhalation exposure to carbon disulfide.

2.2.2 Oral Exposure

Humans are not likely to be exposed to significant quantities of carbon disulfide in food or water. Most information on the effects of oral exposure to carbon disulfide is derived from studies in animals. These studies are summarized in Table 2-2 and Figure 2-2, and the findings are discussed below. All doses are expressed as mg/kg/day.

2.2.2.1 Death

First-hand reports of death from oral exposure to carbon disulfide are very rare. Three case reports cited in Gosselin et al. (1984) indicate that half an ounce (concentration not specified) caused death following ingestion.

In mice, Gibson and Roberts (1972) determined the median oral lethal dose (LD_{50}) over a 24-hour period to be 3,020 mg carbon disulfide/kg. In Wistar rats, a single dose of carbon disulfide administered by gavage at doses up to 632 mg/kg did not cause death, nor were deaths noted in the rats after a 4-week (5 days a week) administration of 253 mg/kg/day carbon disulfide, also by gavage (Hoffmann and Klapperstück 1990). No deaths were noted after a 4-week (5 days a week) administration of 253 mg/kg/day carbon disulfide by gavage to Wistar rats (Hoffmann and Muller 1990).

2.2.2.2 Systemic Effects

The systemic toxicity of carbon disulfide after oral exposure is manifested primarily in the liver, mainly as enzymatic disruptions. Animal data show that hepatic toxicity is potentiated by pretreatment with agents that induce hepatic microsomal enzymes. Cardiovascular effects have also been reported, although these data are limited.

No studies were located regarding respiratory, gastrointestinal, renal, dermal, or ocular effects in humans or animals after oral exposure to carbon disulfide. Information on cardiovascular, hematological, musculoskeletal, hepatic, and body weight effects is presented below. The highest NOAELs and a reliable LOAEL for hepatic and cardiac effects in mice and rats, respectively, for the acute-duration category are recorded in Table 2-2 and plotted in Figure 2-2.

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after oral exposure to carbon disulfide.

Anesthetized male Wistar rats acutely exposed to 632 mg carbon disulfide/kg (0.5 mL/kg) had a significantly reduced occurrence of some cardiac arrhythmias after coronary occlusion by surgical ligation when compared to controls (Hoffman 1987). In addition, the heart rate measured before the procedure was significantly lower in exposed rats than in the controls (Hoffman 1987).

Exposure of anesthetized male rats at a lower gavage dose (253 mg/kg/day) daily for 4 weeks produced a decrease in left ventricular systolic pressure and changes in an electrocardiograph (Hoffmann and Klapperstück 1990). When coronary ligation was performed 1 hour after exposure, these animals showed an earlier appearance of cardiac arrhythmias, delay in acotroitine-induced arrhythmia, and decreased survival. No exposure-related effects occurred at a lower dose level (126 mg/kg). This study also examined the effects of a single gavage administration of carbon disulfide on ECG parameters in anesthetized rats. Changes in ECG were noted at 373 and 506 mg/kg; dose response was noted in QT_c. Heart rate was decreased at 632 mg/kg. No significant changes in contractile force of the left ventricle were noted.

In conscious male Wistar rats administered 506 mg/kg carbon disulfide once by gavage, blood pressure was decreased ($p \leq 0.001$) 5 hours after treatment, but this change was reversible in another 5 hours

TABLE 2-2. Levels of Significant Exposure to Carbon Disulfide - Oral

Key to[a] figure	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
	ACUTE EXPOSURE						
	Systemic						
1	Rat (Wistar)	Once (GO)	Cardio	253 M	506 M (decreased blood pressure)		Hoffman and Klapperstuck 1990
2	Mouse (ddY)	1-14 d 1x/d (GO)	Hepatic		3[b] M (decrease in P-450 and drug metabolizing enzymes)		Masuda et al. 1986
3	Rabbit (New Zealand White)	14 d Gd 9-19 (GO)	Bd Wt			25 F (decrease in body weight gain - 43%)	Jones-Price et al. 1984b
	Neurological						
4	Rat (outbred Zealand White)	10 d Gd 6-15 (GO)		200 F		400 F (hindlimb paralysis)	Jones-Price et al. 1984a
5	Rat (Sprague-Dawley)	Once (G)			300 M (significant decreases in norepinephrine in the midbrain, hypothalamus, and medulla oblongata)		Kanada et al. 1994
6	Rabbit (New Zealand White)	14 d Gd 9-19 (GO)				25 F (hindlimb paralysis)	Jones-Price et al. 1984b
	Developmental						
7	Rat (outbred)	10 d Gd 6-15 (GO)		100 F	200 F (reduced fetal weight)		Jones-Price et al. 1984a

TABLE 2-2. Levels of Significant Exposure to Carbon Disulfide - Oral (continued)

Key to[a] figure	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
8	Rabbit (New Zealand)	14 d Gd 9-19 (GO)				25 F (increased percent resorptions)	Jones-Price et al. 1984b
	INTERMEDIATE EXPOSURE						
	Systemic						
9	Rat (Wistar)	4 wk 5d/wk 1x/d (GO)	Cardio	253 M			Hoffmann and Klapperstuck 1990
			Bd Wt	126 M	253 M (decreased body weight - 10%)		

[a]The number corresponds to entries in Figure 2-2.
[b]Used to derive an acute Minimal Risk Level (MRL) of 0.01 mg/kg/day for carbon disulfide; the LOAEL of 3 mg/kg/day was divided by an uncertainty factor of 300 (3 for use of a minimal LOAEL, 10 for extrapolation from animals to humans, and 10 for interhuman variability).

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); F = female; Gd = gestation day; GO = gavage - oil; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; wk = week(s); x = time(s)

Figure 2-2. Levels of Significant Exposure to Carbon Disulfide – Oral

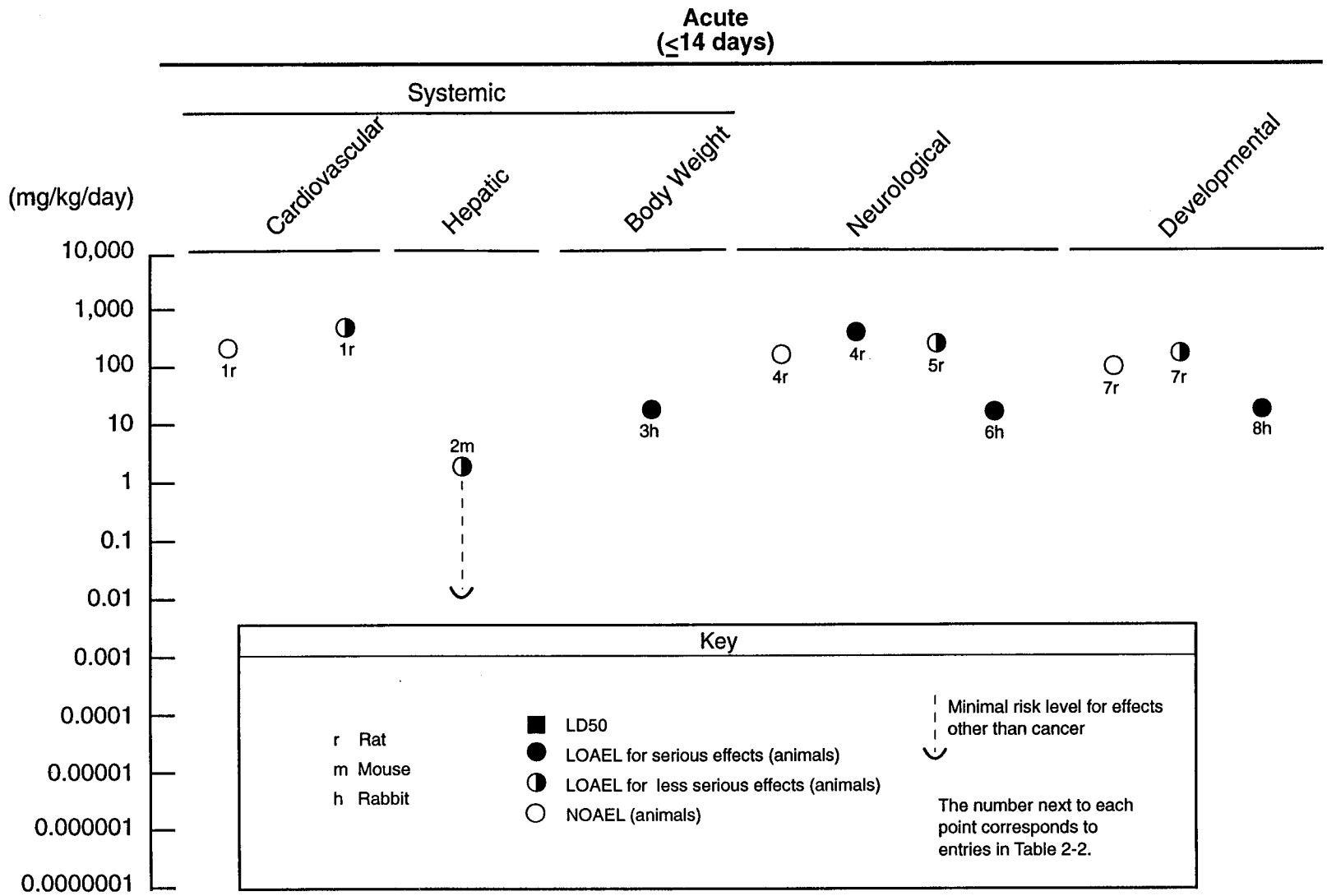
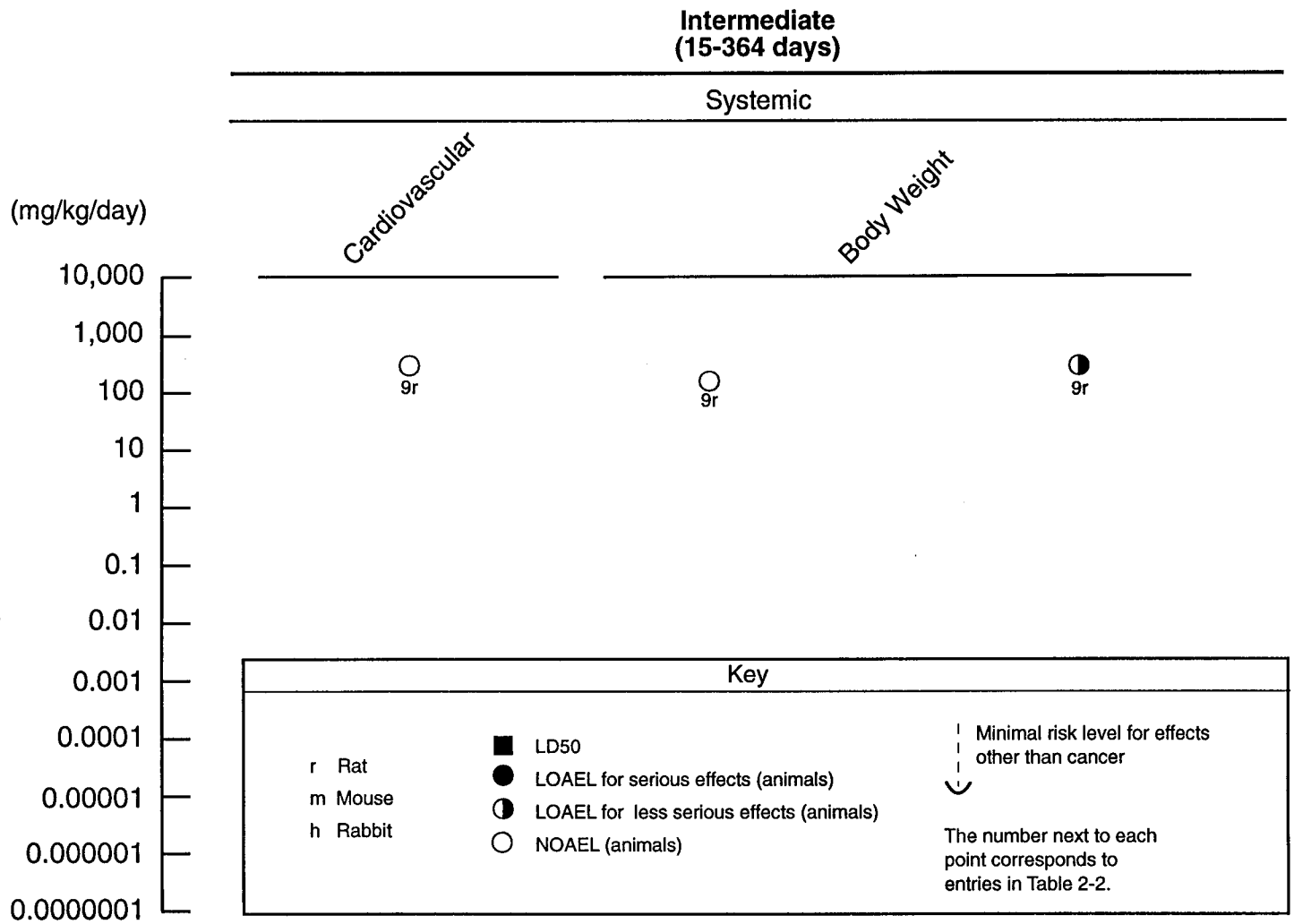


Figure 2-2. Levels of Significant Exposure to Carbon Disulfide – Oral (continued)



(Hoffmann and Klapperstück 1990). The NOAEL was 253 mg/kg. In subacute experiments, no effects on blood pressure or heart rate were observed in rats after receiving carbon disulfide at 126 or 253 mg/kg/day for 4 weeks; however, the study authors did not examine the effects on these parameters at 506 mg/kg/day.

Hematological Effects. No studies were located regarding hematological effects in humans after oral exposure to carbon disulfide.

In a study by Pilarska et al. (1973), rats administered 25 mg carbon disulfide/kg/day for 60 days developed normochromic and normocytic anemia, eosinopenia, and an increase in reticulocyte cell numbers. No changes in leukocyte or platelet numbers were observed, however. No other studies were found on the hematological effects in animals after oral exposure to carbon disulfide.

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans after oral exposure to carbon disulfide.

In rats treated by gavage with carbon disulfide in olive oil at 12.5 mg/kg/day for 1, 2, or 4 weeks, a decreased response of the ancoccygeus muscle to noradrenaline was observed relative to vehicle-treated controls (Gandhi and Venkatakrishna-Bhatt 1993). The response of the muscle to noradrenaline was tested *in vitro* in a muscle bath. The reduction in sensitivity to noradrenaline increased with increasing duration of exposure. The investigators suggested that the mechanism of decreased noradrenaline responsiveness may be a result of a block of calcium influx, a delay of the calcium efflux, an inhibition of the uptake of calcium, a decreased sensitivity to calcium by the muscle, or a combination of these mechanisms.

Hepatic Effects. No studies were located regarding hepatic effects in humans after oral exposure to carbon disulfide.

In animals, oral exposure to carbon disulfide does not appear to cause significant liver toxicity, although the data are limited. Male mice orally exposed to 3-300 mg/kg/day (1-14 days) have shown rapid, reversible, dose-related suppression of hepatic microsomal enzymes (Masuda and Yasoshima 1988; Masuda et al. 1986). An acute-duration MRL of 0.01 mg/kg/day was derived based on dose-dependent decreases in the activities of liver microsomal drug-metabolizing enzymes in mice (Masuda

et al. 1986). The LOAEL for this effect was 3 mg/kg/day. The following enzyme activities were decreased: hydroxylation of aniline, *O*-dealkylation of *p*-nitroanisole, 7-ethoxycoumarin and 7-ethoxyresorufin, *N*-demethylation of *N*,*N*-dimethylaniline, NADPH-cytochrome P-450 reductase activity, and P-450-associated peroxidase activity. In addition, a decrease in cytochrome P-450 content and total heme content was observed. The inhibition of enzyme activities was reversible. There were no effects on the activities of NADH-ferricyanide reductase, NADPH-cytochrome c reductase, flavin-containing monoxygenase, UDP-glucuronyltransferase, glucose-6-phosphatase, heme oxygenase, and glutathione *S*-transferase. Also, the content of cytochrome b_5 was not altered. In rats and mice, single oral doses of 1,263 mg/kg/day have caused necrotic lesions, suppression of microsomal enzymes, fat accumulation, and increased liver weight. These effects are significantly enhanced by pretreatment with a microsomal enzyme inducer such as phenobarbitone (Bond and DeMatteis 1969; Bond et al. 1969; El-Masry et al. 1976; Freundt et al. 1974a). These studies are limited by the use of an unspecified or small number of animals and, in the case of Bond et al. (1969), by the lack of quantitative analysis.

In sheep pretreated with DDT, a dose of 63.2 mg carbon disulfide/kg/day was reported to cause hepatic lesions accompanied by microsomal enzyme suppression and increases in total liver water and electrolytes (Wilkie et al. 1985). This study is of limited value because of its lack of controls to eliminate or account for possible synergistic effects of carbon disulfide and DDT.

Body Weight Effects. No studies were located regarding body weight effects in humans after oral exposure to carbon disulfide.

In a study by Hoffmann and Klapperstuck (1990), male Wistar rats that had been administered 253 mg/kg/day carbon disulfide for 4 weeks by gavage showed a 10% decrease in body weight compared to the controls. In a developmental toxicity study, gestational exposure to 25 mg/kg/day for 10 days produced a 19% decrease in the maternal body weight gain of female New Zealand rabbits (Jones-Price et al. 1984b).

2.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological and lymphoreticular effects in humans or animals after oral exposure to carbon disulfide.

2.2.2.4 Neurological Effects

No studies were located regarding neurological effects in humans after oral exposure to carbon disulfide.

Compared to untreated rats, significant decreases in noradrenaline in the midbrain, hypothalamus, and medulla oblongata were observed in rats 2 hours after they received a single gavage dose of 300 mg carbon disulfide/kg body weight (Kanada et al. 1994). 3,4-Dihydroxyphenylalanine was significantly increased in the midbrain, as was dopamine in the medulla oblongata. In the hippocampus, no change in acetylcholine was observed. Clinical signs were not reported in this study. Hind-limb paralysis was noted in New Zealand rabbits and outbred rats administered 25 and 400 mg/kg/day, respectively, for l0-14 days during gestation (Jones-Price et al. 1984a, 1984b).

2.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans or animals after oral exposure to carbon disulfide.

2.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans after oral exposure to carbon disulfide.

Administration of carbon disulfide by oral gavage at doses up to 150 mg/kg/day to New Zealand white rabbits (gestational days 9-19) and 600 mg/kg/day to albino rats (gestational days 6-15) resulted in signs of maternal toxicity, such as transient hind-limb paralysis and loss of body weight over the period of gestation (Jones-Price et al. 1984a, 1984b). In rabbits, there were statistically significant, dose-related increases in the number of resorptions: mean values of 12, 43, 42, and 61 resorption at 0, 25, 75, and 150 mg/kg/day, respectively. Also, dead fetuses were found: 13%, 33%, 43%, and 62% dead/litter for each respective dose group ($p<0.00l$). Average fetal body weight was decreased significantly in each dose group: 45.5, 45.2, 41.6, 39.5 g/litter for each dose group, respectively. In rabbits, there was a significant increase in the number of malformed fetuses in 150-mg/kg/day (high-dose) animals; however, there was no characteristic pattern of carbon disulfide-related malformations.

Males were affected to a greater extent than females. The teratogenic effect of carbon disulfide appears to be more severe in males at the 150-mg/kg/day dose than in females (when separated by dose, $p<0.036$ for males and $p<0.481$ for females), whereas the percentage of live fetuses and the average fetal body weight are not sex-dependent. In contrast, rats exhibited only decreased average fetal weight at doses greater than 200 mg/kg/day (moderate dose). No increase in resorption, fetal deaths, or malformations was found in carbon disulfide-treated rats.

The highest NOAEL and a reliable LOAEL for developmental effects in rats and rabbits are recorded in Table 2-2 and are plotted in Figure 2-2.

2.2.2.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or animals after oral exposure to carbon disulfide.

Genotoxicity studies are discussed in Section 2.5.

2.2.2.8 Cancer

No studies were located regarding cancer in humans or animals after oral exposure to carbon disulfide.

2.2.3 Dermal Exposure

2.2.3.1 Death

No studies were located regarding death in humans or animals after dermal exposure to carbon disulfide.

2.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, or ocular effects in humans or animals following dermal exposure to carbon disulfide.

Dermal Effects. Dermal contact with carbon disulfide is important in the occupational setting (Dutkiewicz and Baranowska 1967). Viscose rayon workers have been reported to develop serious blisters that progressed to hemorrhagic blisters covered by a thin membrane. These blisters appeared on the fingers in spite of wearing rubber gloves (Hueper 1936).

The implication of carbon disulfide as the causative agent is supported by experiments in rabbits whose ears were dermally exposed to carbon disulfide. Blisters similar to those found in the rayon workers developed on the animals' ears despite protective covering (Hueper 1936).

No studies were located regarding the following health effects in humans or animals after dermal exposure to carbon disulfide:

2.2.3.3 Immunological and Lymphoreticular Effects

2.2.3.4 Neurological Effects

2.2.3.5 Reproductive Effects

2.2.3.6 Developmental Effects

2.2.3.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.5.

2.2.3.8 Cancer

No studies were located regarding cancer in humans or animals after dermal exposure to carbon disulfide.

2.3 TOXICOKINETICS

The available data from human and animal studies indicate that carbon disulfide is extensively and rapidly absorbed via inhalation, oral, and dermal routes. Absorbed carbon disulfide is distributed throughout the body. Because of its lipophilic nature, its distribution is greatest in organs such as the brain and liver where it is metabolized to thiocarbamates. Carbon disulfide is metabolized by cytochrome P-450 to an unstable oxygen intermediate which either spontaneously degrades to atomic sulfur and carbonyl sulfide or hydrolyzes to form atomic sulfur and monothiocarbonate. Carbonyl

sulfide is converted to monothiocarbonate by carbonic anhydrase. Monothiocarbonate degrades to generate carbonyl sulfide or forms carbon dioxide and hydrogen sulfide. Unlike in animals, oxidation of sulfur to inorganic sulfate does not contribute significantly to the metabolism of carbon disulfide in humans. Despite the differences in the metabolism of carbon disulfide between animals and humans, dithiocarbamates are the common metabolites formed in these species after reaction with amino acids. These metabolites contribute in part to the neurotoxic effects of carbon disulfide.

The kidneys are the primary route of excretion of carbon disulfide metabolites. Conjugation of carbon disulfide or carbonyl sulfide with endogenous glutathione results in formation of thiozolidine-2-thione-4-carboxylic acid and 2-oxythiazolidine-4-carboxylic acid, respectively, which are excreted in the urine. The unmetabolized carbon disulfide is excreted unchanged in the breath, and small amounts (<l%) have been detected in the urine.

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

Studies conducted on human subjects reported rapid and extensive absorption of inhaled carbon disulfide. Rapid absorption was demonstrated in a study conducted on volunteers exposed to 17-51 ppm for l-4 hours (Teisinger and Soucek 1949). The amounts of carbon disulfide retained in the body and excreted by the lungs and kidneys were determined by measuring the carbon disulfide in inspired and expired air, blood, and urine during and after completion of the experiment until it disappeared from the urine and blood. About 80% of the inhaled carbon disulfide was retained during the first 15 minutes of exposure which decreased to about 40% after 45 minutes and remained at that level for the rest of the exposure period. The degree of retention did not depend on the exposure concentration. Only 5% of the retained carbon disulfide at the end of the exposure period was subsequently eliminated in the exhaled air. About 0.06% of the retained carbon disulfide was excreted unchanged in the urine and was detectable 24 hours after exposure. In another retention study involving exposure to vapor for an unspecified period (Soucek 1957), about 10-30% of the retained carbon disulfide was exhaled, and less than 1% was excreted in urine as carbon disulfide. The concentration of inhaled carbon disulfide was not reported. About 70-90% was metabolized.

Studies in animals indicate that carbon disulfide is rapidly absorbed following inhalation exposure. Absorption of carbon disulfide was studied by evaluating pulmonary and urinary excretion of carbon disulfide during and after exposure. Studies in rabbits indicate that an equilibrium concentration of carbon disulfide is reached after inhalation exposure to 20-150 ppm for 1.5-2.0 hours (Toyama and Kusano 1953). About 70-80% of the inhaled carbon disulfide was absorbed. After termination of exposure, 15-30% of the absorbed carbon disulfide was excreted through the lungs and less than 0.1% by the kidneys. In dogs exposed to 25-60 ppm carbon disulfide, equilibrium concentrations in blood were attained after 0.5-2.0 hours (McKee et al. 1943). Desaturation of blood carbon disulfide was almost complete within the first 30-60 minutes after exposure. Approximately 8-13% of the retained carbon disulfide was exhaled, less than 0.5% was excreted in the urine, and none was excreted in the feces. Excretion in the urine occurred within 2 hours of exposure. Freundt et al. (1975) observed that an equilibrium concentration of carbon disulfide in blood was attained after exposure of rats to 400 ppm carbon disulfide for 1 hour. Equilibrium was reached in liver and blood between 1 and 8 hours after exposure. Elimination of free carbon disulfide from these tissues was rapid, with an estimated half-life in the blood of 35 minutes and in the liver of approximately 1 hour.

The data presented above indicate that carbon disulfide is absorbed by humans and animals following inhalation exposure and reaches equilibrium rapidly (0.5-8 hours) across a wide range of doses and exposure durations.

2.3.1.2 Oral Exposure

No studies were located regarding absorption of carbon disulfide following oral exposure of humans. In rats, intragastric administration of 10 mg/kg ^{14}C-carbon disulfide resulted in exhalation of 63% of the dose within 4 hours as unchanged carbon disulfide (DeMatteis and Seawright 1973). It is evident from these results that a large fraction of orally administered carbon disulfide is absorbed by rats.

2.3.1.3 Dermal Exposure

Dermal exposure of humans to aqueous solutions of carbon disulfide resulted in significant absorption through the skin. A series of experiments were performed to investigate the rate of absorption of carbon disulfide by immersion of the hand in aqueous solutions of increasing concentrations (0.33-1.67 g/L) for 1 hour (Dutkiewicz and Baranowska 1967). Absorption was calculated indirectly

by determining carbon disulfide elimination by the lung or directly by measuring carbon disulfide concentration in the solutions before and after immersion of the hand. Rates of absorption of carbon disulfide, determined from analysis of the solutions, ranged from 0.232 to 0.789 mg/cm^2/hour and were about 10 times higher than rates calculated from lung excretion of carbon disulfide. In the former case, 25% of the absorbed dose was exhaled in the desaturation period; in the latter, only 3% was eliminated in the expired air. These findings suggest that carbon disulfide excretion varies with the route of absorption. This study provided only brief details of the experimental procedure, and therefore factors other than absorption through the skin (e.g., evaporation) may have accounted for the reduced carbon disulfide concentration noted at the end of the experimental period. Nevertheless, these results suggest that rapid absorption of carbon disulfide can occur in humans through skin. Occupational exposure of persons with pathological skin conditions has also been noted to increase the dermal absorption of carbon disulfide (Drexler et al. 1995a).

The limited information available on skin absorption in animals indicates that carbon disulfide is appreciably absorbed. Exposure of rabbit skin to high concentrations of the vapor (800 ppm and above) for 1 hour resulted in detectable amounts of carbon disulfide in the breath (Cohen et al. 1958). A linear relationship was noted between the dermal exposure concentration and the amount of carbon disulfide exhaled. No detectable carbon disulfide was found in the breath of rabbits exposed to 150 ppm vapor by skin contact for 6 hours (Cohen et al. 1958).

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

Absorbed carbon disulfide is taken up by the blood (McKee et al. 1943) and is distributed throughout the body (Brieger 1967). Because of the lipophilic nature of carbon disulfide, distribution is greatest to lipid-rich tissues and organs such as the brain and liver where it is metabolized to dithiocarbamate (Santodonato et al. 1985). Milk from nursing mothers occupationally exposed to carbon disulfide was found to contain an average of 12.3 μg carbon disulfide/l00 mL (Cai and Bao 1981). Exposure concentrations of carbon disulfide ranged from 9.3 to 21.1 ppm for a 6.5-hour period. Exposure to 7.4-40 ppm for a shorter duration (2-4 hours) resulted in a lower average milk concentration of 6.8 μg/l00 mL.

The distribution of carbon disulfide following inhalation exposure has been studied in rabbits and rats (Toyama and Kusano 1953). In rabbits, blood equilibrium concentrations of carbon disulfide were reached after exposure to 20-150 ppm for 1.5-2.0 hours. In rats exposed to 60-350 ppm carbon disulfide, distribution was primarily to the brain, kidney, and liver. In contrast to rabbits, blood equilibrium concentrations for various carbon disulfide exposures in rats were not determined. Although carbon disulfide was rapidly eliminated from rat tissues during the first 6-8 hours after exposure, low concentrations of carbon disulfide were still detected in the tissues 20 hours after exposure. A separate study reported that equilibrium concentrations of carbon disulfide in blood were attained in dogs after 0.5-2.0 hours of exposure to 25-60 ppm carbon disulfide (McKee et al. 1943). Desaturation was largely complete within the first 30-60 minutes after inhalation exposure. Anesthetized male Sprague-Dawley rats exposed to 640 ppm carbon disulfide had an exponential increase in carbon disulfide in the blood which reached an apparently steady state after 90 minutes of exposure. After discontinuation of exposure, the blood concentration decreased rapidly, with elimination half-lives reported to be 6 and 85 minutes for the fast and slow components, respectively. In all tissues except fat, the carbon disulfide concentration approached steady state within 4-5 hours of exposure. Loss of free carbon disulfide was rapid from all tissues except the liver and kidneys, which retained 25% and 29%, respectively, at 8 hours postexposure (McKenna and DiStefano 1977a).

Inhalation exposure of pregnant mice to carbon disulfide during gestation resulted in rapid absorption and distribution of carbon disulfide and its metabolites in embryonic and fetal tissues within 1 hour (Danielsson et al. 1984). Pregnant mice were exposed via inhalation to 25 microcuries (μCi) ^{35}S- or ^{14}C-carbon disulfide for 10 minutes on day 9, 14, or 17 of gestation. The levels of ^{35}S-labelled metabolites in the embryonic neuroepithelium were higher in the fetal brain than in the maternal brain during early gestation (day 9). The concentrations in the fetal brain, eyes, and skeleton exceeded that of other fetal organs during mid-gestation (day 14). In late gestation (day 17), the levels in the fetal and maternal brain were relatively low, but high uptake of radioactivity was seen in the placenta, fetal blood, liver, and eyes. During early gestation, the distribution of ^{14}C-labelled metabolites was similar to that of ^{35}S-labelled metabolites with an immediate higher uptake in the embryo (including neuroepithelium) than in the maternal serum. On days 14 and 17 of gestation, radioactivity was present in the ventricle of the fetal brain. High levels were detected in the fetal liver and blood at late gestation (day 17). In contrast to ^{35}S-labelled metabolites, ^{14}C-labelled metabolites were retained longer (up to 24 hours) in the fetal brain and liver. High concentrations of ^{14}C-labelled metabolites were also seen in the fetal urinary tract. Thus, the distribution pattern varied with the age of the

conceptus and also with the radiolabel of carbon disulfide. These results indicate that carbon disulfide and its metabolites pass through the placenta at all stages of gestation and localize selectively in various tissues of the body.

2.3.2.2 Oral Exposure

No studies were located regarding distribution of carbon disulfide in humans or animals following oral exposure.

2.3.2.3 Dermal Exposure

No studies were located regarding distribution of carbon disulfide in humans or animals following dermal exposure.

2.3.2.4 Other Routes of Exposure

The distribution of free carbon disulfide and bound carbon disulfide liberated by acid hydrolysis was investigated in the tissues of white rats after a large, single subcutaneous dose (approximately 361 mg/kg) of carbon disulfide (Bartonicek 1957, 1959). Results of these studies indicate that following absorption, free carbon disulfide is rapidly removed from the blood and tissues. Negligible blood levels were present 11 hours after the dose was administered (Bartonicek 1957, 1959). Initially, free carbon disulfide accumulated in the blood, adrenals, and brain, but levels in the organs rapidly decreased, and only very small amounts were present after 10-16 hours.

A similar rapid reduction of free carbon disulfide levels in the blood was noted when radiolabelled ^{35}S-carbon disulfide was administered parenterally to guinea pigs (Strittmatter et al. 1950). About 20-50% of intracardially injected ^{35}S-carbon disulfide was retained; the amount of material retained depended on the concentration of dose administered. The largest amount of radiolabel appeared in the liver (0.43 μg) and the least amount in the brain (0.05 μg) at 1.5 hours following injection. Only 10% of the labelled compound remained in the tissues after 48 hours. Urinary and fecal excretion was not reported. In guinea pigs exposed to carbon disulfide vapors (13.6-25.7 ppm), the brain and blood contained more ^{35}S-label relative to the liver. Forty-eight hours later, 30-50% of ^{35}S-label remained in the tissues such as blood, liver, brain, kidney, and skin. The urinalyses revealed that urinary ^{35}S-label

was about 30% of the retained sulfur, with about 85% or 90% of it appearing in the first 24-hour output, the larger part of the metabolized material in the urine being excreted as inorganic sulfate. The feces contained about 5-15% metabolized ^{35}S-label, the amount of which increased with the increasing dose of carbon disulfide.

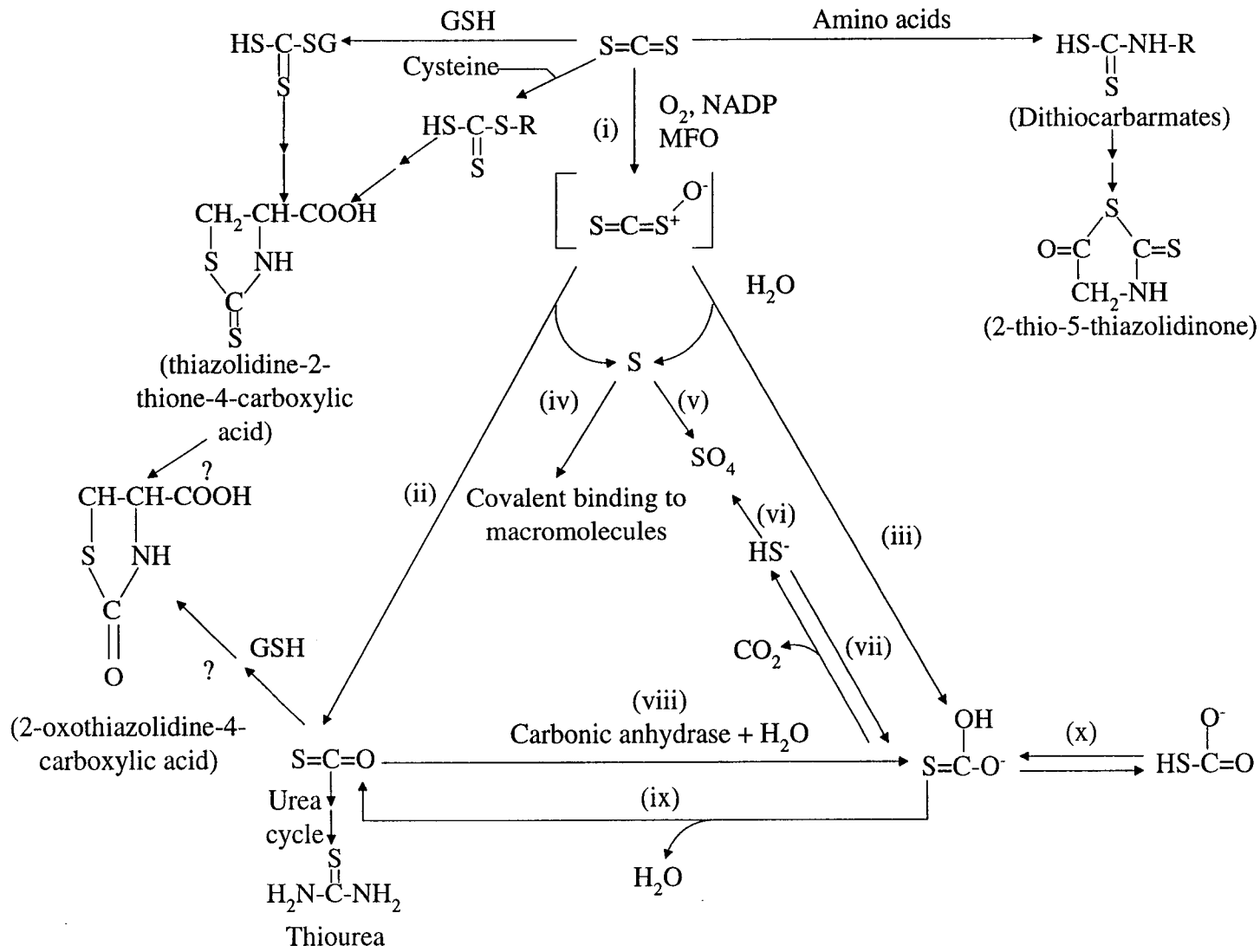
Only metabolites of carbon disulfide were found 3 hours after a dose of ^{14}C- or ^{35}S-labeled carbon disulfide was intraperitoneally administered (Snyderwine and Hunter 1987). Distribution varied with the age of the rat and the radiolabel injected. Following intraperitoneal administration of ^{14}C-carbon disulfide, 4-9% of the dose was metabolized to carbon dioxide depending on age. Significantly more carbon disulfide was metabolized to carbon dioxide by 30- and 40-day-old rats than by 1-20-day-old rats. The biotransformation products of carbon disulfide which were covalently bound remained in tissues from rats of all ages. Twenty-four hours after dosing with ^{35}S-labeled carbon disulfide, up to 13 times more labeled metabolites were covalently bound in organs from l-day-old rats than in similar organs from 40-day-old rats.

The data presented above indicate that the absorbed carbon disulfide is rapidly distributed via blood to other tissues irrespective of the route of exposure.

2.3.3 Metabolism

Limited information is available on the biotransformation of carbon disulfide in humans, and the metabolic products of carbon disulfide are not completely known. In animals and humans the proposed metabolic pathways involved in the metabolism of carbon disulfide (Beauchamp et al. 1983) are depicted in Figure 2-3, reactions i-x. Reaction i has been demonstrated in *in vivo* animal studies and in *in vitro* assays. Reactions ii-v are proven by *in vitro* studies, while products of reactions vi-ix are the results of proposed metabolic pathways of carbon disulfide in animals and humans. Carbon disulfide is metabolized by cytochrome P-450 to an unstable oxygen intermediate (reaction i). The intermediate may either spontaneously degrade to atomic sulfur and carbonyl sulfide (reaction ii) or hydrolyze to form atomic sulfur and monothiocarbonate (reaction iii). The atomic sulfur generated in these reactions may either covalently bind to macromolecules (reaction iv) or be oxidized to products such as sulfate (reaction v). The carbonyl sulfide formed in reaction ii may be converted to monothiocarbonate by carbonic anhydrase (reaction viii). Monothiocarbonate may further spontaneously degrade in reaction ix, regenerating carbonyl sulfide or forming carbon dioxide and

FIGURE 2-3. Proposed Metabolic Pathways for Carbon Disulfide*



***Source: Beauchamp et al. 1983**

sulfide bisulfide ion (HS^-) (reaction vii). The HS^- formed in reaction vii can subsequently be oxidized to sulfate or other nonvolatile metabolites (reaction vi).

Dithiocarbamates are the products of the reaction of carbon disulfide with amino acids (Brieger 1967). *In vitro* studies demonstrated that carbon disulfide readily combines with the amino acids in human blood, the half-life of this reaction being approximately 6.5 hours (Soucek 1957). Thiocarbamide has been found in the urine of exposed workers (Pergal et al. 1972b). After inhalation exposure of male subjects, up to 90% of the retained carbon disulfide was metabolized while the remainder was eliminated unchanged by various routes (McKee et al. 1943). High levels of thiocarbamide and trace amounts of 2-thio-5-thiazolidinone were identified by chromatographic analysis of the urine of workers exposed to carbon disulfide by inhalation (Pergal et al. 1972a, 1972b). Van Doorn et al. (1981a, 1981b) reported conjugation of carbon disulfide or carbonyl sulfide with endogenous glutathione to yield thiazolidine-2-thione-4-carboxylic acid and 2-oxythiazolidine-4-carboxylic acid, respectively. High concentrations (approximately 320 mM) of thiazolidine-2-thione-4-carboxylic acid (TTCA) were detected in the urine of women exposed to approximately 32 ppm (100 mg/m^3) carbon disulfide through inhalation (refer to Figure 2-3).

In contrast to the results obtained in animals, oxidation to inorganic sulfate does not appear to contribute significantly to the metabolism of carbon disulfide in humans. A marked increase in inorganic sulfate excretion in the urine was noted in a case study of a young worker with signs of carbon disulfide poisoning because of exposure to high levels of the vapor; no increase was noted in the amount of inorganic sulfate excreted in the urine (Djerassi and Lumbroso 1968). However, exact dose, mode of exposure, and duration were not presented in the study.

Carbon disulfide is oxidized by the liver mixed-function oxidase (MFO) system to carbonyl sulfide, which then undergoes further desulfurization, releasing elemental sulfur. This reaction has been shown to occur in vitro (Dalvi et al. 1974; DeMatteis 1974). *In vivo* studies in rats using ^{14}C-labelled carbon disulfide demonstrated that significant amounts (80%) of $^{14}CO_2$, are exhaled after exposure to carbon disulfide. Following intraperitoneal administration of approximately 100 mg carbon disulfide/kg, about 5% of the total dose was excreted in the breath as carbon dioxide. This amount was increased to 13% in animals pretreated with phenobarbital to induce liver microsomal enzymes (DeMatteis and Seawright 1973). Snyderwine and Hunter (1987) found that 4-9% of an intraperitoneally administered dose of ^{14}C-carbon disulfide was excreted as $^{14}CO_2$ in expired air, with 30- and 40-day-old rats

excreting more (9% versus 4%) $^{14}CO_2$, than 1-20-day-old rats. This was attributed to the increased hepatic MFO of carbon disulfide to carbon dioxide in 30-40-day-old rats.

The metabolic formation of carbonyl sulfide from carbon disulfide was confirmed in an in *vivo* study (Dalvi and Neal 1978). After intraperitoneal injection of ^{14}C-carbon disulfide in nonpretreated rats, carbonyl sulfide was excreted by the lung in greater quantities than carbon dioxide. Pretreatment with phenobarbital, however, resulted in a greater amount of excretion of carbon dioxide than carbonyl sulfide. In both experiments, excretion of ^{14}C-carbonyl sulfide and carbon dioxide accounted for 14-43% of the total administered radioactivity, with about twice as much carbon dioxide. These results indicate that phenobarbital treatment caused induction of cytochrome P-450 which catalyzed the conversion of carbon disulfide to carbonyl sulfide faster in pretreated rats than in rats not pretreated with phenobarbital. The role of the cytochrome P-450 monooxygenase system in catalyzing carbonyl sulfide formation was also confirmed by *in vitro* studies (Dalvi et al. 1974, 1975). The rate of carbonyl sulfide formation was NADPH-dependent and increased with microsomes obtained from phenobarbital-treated rats.

In a study designed to examine the effect of P-450 induction on the metabolism of carbon disulfide to TTCA, rats were treated with nothing, ethanol, phenobarbital, 3-methylcholanthrene, or phenobarbital and ethanol before being exposed to carbon disulfide at 50 ppm for 6 hours (Kivisto et al. 1995). After 7 days the pretreatment regimens were repeated in the same rats, and the rats were again exposed to carbon disulfide at 500 ppm for 6 hours. None of the inducers had any effect on urinary excretion of TTCA. About 7.6% and 2.3% of the dose was excreted as TTCA at 50 and 500 ppm, respectively, suggesting saturation. However, the investigators speculated that saturation may not have occurred because the physical activity level of the rats was reduced at 500 ppm suggesting that carbon disulfide uptake at 500 ppm may also have been reduced because of the lowered respiratory rate. They also note that the saturation observed in rats is not likely to occur in humans at the prevailing occupational exposure concentrations. Saturation of TTCA production was observed in an oral study in rats (Kivisto et al. 1995). In rats treated with a single gavage dose of 1, 10, 30, or 100 mg/kg, 4.6%, 2.4%, 1.7%, and 0.8%, respectively, of the dose was excreted in the urine as TTCA.

The effect of P-450 induction or glutathione depletion on carbon disulfide metabolism to TTCA in rats following oral exposure has also been studied (Kivisto et al. 1995). The rats were pretreated with nothing, acetone, phenobarbital, 3-methylcholanthrene, or three inhibitors of glutathione production,

namely phorone, diethylmaleate, or buthionine sulfoximine, before being given a single gavage dose of carbon disulfide at 26-34 mg/kg. Phenobarbital decreased the output of TTCA by 21% during the first 12 hours of the urine collection. None of the other P-450 inducers had any effects on TTCA excretion, and the investigators suggested that the effect of phenobarbital may have been a result of cytochrome P-450 aggregation. Buthionine sulfoximine, an inhibitor of glutathione production, reduced the total output of TTCA by about 40%. Phorone and diethylmaleate pretreatment, which transiently reduce glutathione, decreased TTCA excretion.

2.3.4 Excretion

2.3.4.1 Inhalation Exposure

Following inhalation exposure, the primary route of excretion of unmetabolized carbon disulfide in humans is exhalation. In one study it was estimated that 6-10% of the carbon disulfide that was taken up was excreted by the lungs (McKee et al. 1943). In a study conducted on humans, carbon disulfide levels in the exhaled breath decreased rapidly on cessation of exposure (Soucek 1957). The excretion by the lung accounted for 10-30% of the absorbed carbon disulfide. Less than 1% was excreted unchanged in the urine. The remaining 70-90% of the dose was metabolized. The details regarding carbon disulfide exposure levels were not available. A correlation was established between carbon disulfide exposure of rayon workers and urinary excretion of a metabolite or metabolites that catalyzed the reaction of iodine with sodium azide (Djuric 1967). This test indicated exposures to carbon disulfide above 16 ppm but failed to identify specific urinary metabolites. The failure to detect carbon disulfide exposure below 16 ppm may be because of interference with the reaction by dietary sulfur containing compounds.

In dogs exposed to 25-60 ppm carbon disulfide for 0.5-2.0 hours, approximately 8-13% of the carbon disulfide that was taken up was exhaled; less than 0.5% was excreted in the urine (McKee et al. 1943). Experimental details and control information are limited in this study. Inhalation exposure of rabbits to 20-150 ppm carbon disulfide for 1.5-2 hours resulted in excretion of 15-30% of the absorbed carbon disulfide via the lung and less than 0.1% by the kidney after termination of exposure (Toyama and Kusano 1953).

In guinea pigs, carbon disulfide metabolites are excreted as inorganic sulfur compounds in the urine (Strittmatter et al. 1950). Inhalation exposure to 14 ppm ^{35}S-carbon disulfide for 8 hours or to 26 ppm ^{35}S-carbon disulfide for 40 hours resulted in excretion of the administered dose mainly in the urine (63%) and expired air (30%) within 48 hours of exposure. The metabolized material was excreted in the urine predominantly in the form of inorganic sulfur compounds; some organosulfur derivatives were also present. Most of the unmetabolized carbon disulfide was excreted in the expired air.

The studies discussed above indicate that the lungs are the primary route of excretion of unmetabolized carbon disulfide in humans and animals exposed by inhalation, whereas the kidneys are the primary route of excretion of carbon disulfide metabolites.

2.3.4.2 Oral Exposure

No studies were located regarding excretion of carbon disulfide in humans after oral exposure.

Rats administered 10 mg ^{14}C-carbon disulfide/kg by gavage excreted 63% of the dose as unchanged carbon disulfide in the breath (DeMatteis and Seawright 1973).

2.3.4.3 Dermal Exposure

Following dermal exposure of humans to aqueous solutions of carbon disulfide of increasing concentrations (0.33-1.67 g/L) for 1 hour, only 3% of the absorbed carbon disulfide was eliminated by the lungs (Dutkiewicz and Baranowska 1967). For details and study limitations, see Section 2.3.1.3.

Exposure of rabbit skin to high concentrations of carbon disulfide vapor (800 ppm and above) for 1 hour resulted in detectable amounts of carbon disulfide in the breath of animals (Cohen et al. 1958). A linear relationship was noted between the exposure concentration and the amount of carbon disulfide in the exhaled breath.

2.3.4.4 Other Routes of Exposure

Appreciable amounts of absorbed carbon disulfide are excreted unchanged in breath regardless of the route of exposure (refer to Sections 2.3.4.1, 2.3.4.2, and 2.3.4.3). Small amounts of carbon disulfide

are excreted in the sweat and saliva of exposed individuals. In mice injected intraperitoneally with 30-42 μg of ^{35}S-carbon disulfide, about 13-23% of the radiolabel was excreted via the lung (Strittmatter et al. 1950). Rats receiving 10 mg ^{14}C-carbon disulfide/kg by intraperitoneal injection excreted about 70% of the dosed material as unchanged carbon disulfide in the breath (DeMatteis and Seawright 1973). Rats receiving 19 mg/kg ^{14}C-carbon disulfide intraperitoneally excreted 58-83% free carbon disulfide in expired air in the 3 hours following dosing (Snyderwine and Hunter 1987). Younger rats expired significantly more free carbon disulfide than older rats. In another study (Dalvi and Neal 1978), intraperitoneal administration of ^{14}C-carbon disulfide to rats resulted in excretion of carbonyl sulfide by the lungs in greater quantities than carbon dioxide. Pretreatment of rats with phenobarbital, however, resulted in a greater amount of excretion of carbon dioxide than carbon disulfide. In both experiments, excretion of ^{14}C-carbonyl sulfide and carbon dioxide accounted for 14-43% of the total administered radioactivity, with about twice as much carbon dioxide.

2.4 MECHANISMS OF ACTION

Despite the apparent differences between animals and humans in the metabolism of carbon disulfide, dithiocarbamates are the common metabolites formed. These may in part account for the neurotoxic effects of carbon disulfide. Formation of dithiocarbamates has been demonstrated in both *in vitro* and *in vivo* studies in these species. Metabolism studies in animals clearly indicate that carbon disulfide is metabolized by two distinctly different pathways (see Figure 2-3): it can form dithiocarbamates and glutathione conjugates, or it can be catalyzed by the monooxygenase system to generate reactive sulfur. However, the relative contributions of each of these metabolic pathways to the development of the acute and chronic toxicity of carbon disulfide remain to be determined. Both metabolic pathways suggest several potential mechanisms of toxicity. Formation of dithiocarbamates may in part account for peripheral neurotoxicity. Furthermore, the nonenzymatic reaction of carbon disulfide with free amino groups suggests a potential interaction with biological macromolecules such as proteins and nucleic acids. In contrast, the formation or generation of reactive sulfur may inhibit the microsomal monooxygenase system and disturb the metabolism of other endogenous and exogenous compounds.

In the paragraphs that follow, the two metabolic pathways are discussed in detail. The relative importance of these two pathways is also discussed.

Carbon disulfide combines readily with the amine groups of amino acids to produce dithiocarbamates, which are water-soluble metabolites. Such reactions have been demonstrated with free amino groups in serum *in vivo* (Cohen et al. 1958). The formation of acid-labile carbon disulfide, readily destroyed at low pH, was also consistent with significant *in vivo* formation of dithiocarbamate metabolites (McKenna and DiStefano 1977a; Snyderwine and Hunter 1987). Following absorption of inhaled carbon disulfide by the lung, free carbon disulfide is distributed to various tissues where it is either eliminated, primarily by the lung, or further metabolized to acid-labile carbon disulfide metabolites. The formation of acid-labile carbon disulfide metabolites may continue to increase at steady-state concentrations of carbon disulfide as long as free carbon disulfide is available to the tissue and amine substrates are available. This was demonstrated in rats exposed to 640 ppm carbon disulfide for 8 hours in which acid-labile carbon disulfide metabolites continued to accumulate in several tissues after steady-state levels of carbon disulfide were reached (McKenna and DiStefano 1977a).

The formation of the carbon disulfide metabolite, dithiocarbamate, may explain the mechanism of carbon disulfide-induced neurotoxic effects. For example, McKenna and DiStefano (1977b) found a decrease in the activity of copper-requiring enzyme, dopamine-β-hydroxylase, in response to increased inhalation exposure to carbon disulfide (0.1-2.0 mg/L for 8 hours). The effect of carbon disulfide was attributed to the formation of dithiocarbamates, which complex with copper, since in vitro inhibition of purified dopamine-β-hydroxylase by carbon disulfide was dependent on preincubation with amines capable of dithiocarbamate formation (McKenna and DiStefano 1977b). The inhibition of dopamine-β-hydroxylase decreased progressively with increasing Cu^{++} concentration, and equimolar concentrations of Cu^{++} and inhibitor were without effect, suggesting that the inhibition occurred through the binding of enzymic copper.

An alternative mechanism proposed to explain the neurotoxic effect of carbon disulfide is the formation of a dithiocarbamate derivative of pyridoxamine, a form of vitamin B_6, with carbon disulfide (Vasak and Kopecky 1967). Investigators postulated that transaminases and amine oxidases would be inhibited because these enzymes require the pyridoxamine phosphate form of vitamin B_6, as a cofactor. In subsequent *in vivo* studies, evidence of altered tryptophan metabolism (consistent with an inhibition of B_6-requiring enzymes) appeared to support this hypothesis (Abramova 1966). Furthermore, supplementation of the diet with vitamin B_6, delayed some of the neurotoxic effects of carbon disulfide (Teisinger 1974). However, continued research could not detect any change in the tissue content of vitamin B_6 in rats after chronic exposure to carbon disulfide (Okayama et al. 1988), and in a later

study the authors suggested that the abnormalities in tryptophan metabolism caused by exposure to carbon disulfide were not related to vitamin B_6 deficiency (Okayama et al. 1988). In this instance, they studied the activities of enzymes in the kynurenine pathway in rats chronically exposed to carbon disulfide. Increased activities of L-tryptophan-2,3-dioxygenase, kynurenine-3-hydroxylase, and kynureninase in the high-concentration group (800 ppm) were found; this may indicate activation of the kynurenine pathway upon carbon disulfide exposure rather than vitamin B_6 deficiency (Okayama et al. 1988).

The effects of carbon disulfide on the microsomal drug metabolizing system were demonstrated in rats by Freundt et al. (1975). Exposure of rats by inhalation to low concentrations of carbon disulfide (20-400 ppm for 8 hours) inhibited microsomal drug metabolism. This was reflected by increased hexobarbital sleep times. Pretreatment of rats with SKF-525A, an inhibitor of cytochrome P-450-mediated metabolism, reduced the liver damage from carbon disulfide in phenobarbital-pretreated animals (Bond and DeMatteis 1969).

Valentine et al. (1993) have shown that the initial dithiocarbamate protein adduct decomposes to isothiocyanate derivatives which then react with protein nucleophiles resulting in crosslinking. The crosslinking of protein in the nerve axons to cause their ultimate degeneration is correlated with the crosslinking of spectrin, a blood cell membrane protein. This suggests that the latter can be used as a biomarker of adverse effect of nerve damage.

Acute intraperitoneal injections of 504 mg/kg carbon disulfide for 3 consecutive days in male rats caused a significant decrease in aminopeptidase activity in the thalamus and cerebellum of the brain (de Grandarias et al. 1992). The role of aminopeptidase activity in carbon disulfide neurotoxicity was postulated.

It has been postulated that carbon disulfide cardiotoxicity may be mediated by disruption of the energy supply in the heart (Klapperstuck et al 1991). The mechanism for carbon disulfide acceleration of arteriosclerotic plaque formation involves direct injury to the vessel epithelium and changes in lipid metabolism.

It has been postulated that the reduced thyroid activity in workers exposed to carbon disulfide may be related to a central involvement of catecholamine metabolism (Cavalleri et al. 1978).

2.5 RELEVANCE TO PUBLIC HEALTH

There are substantial data available on which to base conclusions regarding the potential health effects of carbon disulfide exposure in residents near hazardous waste sites and occupationally exposed individuals. The principal adverse health effects noted in humans exposed via inhalation are neurotoxic and cardiovascular effects (Aaserud et al. 1988, 1990; Egeland et al. 1992; Hernberg et al. 1971; Hirata et al. 1992a; Lancranjan 1972; Ruijten et al. 1990, 1993; Sikora et al. 1990; Tolonen et al. 1979; Vanhoorne et al. 1992a). Most of the human data are derived from occupational studies. Although these are somewhat limited by the difficulties in estimating the exact dose for individuals and by the fact that most industrial environments have multiple types of exposures, these data are consistent with effects noted in experimental animal studies. In addition, the toxicokinetic studies and the derivations of mechanisms of action are consistent with these observations. Specific health effects are discussed in greater detail below.

Minimal Risk Levels for Carbon Disulfide

A User's Guide has been provided at the end of the profile (see Appendix A) to aid in the interpretation of MRLs.

Inhalation

- An MRL of 0.3 ppm was derived for chronic exposure to carbon disulfide. This MRL was derived based on peripheral neuropathy (reduced motor nerve conduction velocity) in humans after prolonged occupational exposure to carbon disulfide. A LOAEL of 7.6 ppm was established for this effect. Although a dose response was achieved, the effect was considered minimal since the reductions in motor nerve condition velocity were within a range of clinically normal values. This concentration was divided by an uncertainty factor of 30 (3 for use of a LOAEL, 10 for human variability) to yield the calculated MRL of 0.3 ppm.

No acute-duration or intermediate-duration inhalation MRLs were derived since potential MRL values would not have been as protective of human health as the chronic-duration MRL. In addition, there is greater confidence in the chronic-duration MRL since it is based on human data.

Oral

- An MRL of 0.01 mg/kg/day was derived for acute exposure to carbon disulfide. This MRL was derived based on the inhibition of enzyme activities, specifically decreases in the activities of several hepatic microsomal cytochrome P-450-dependent drug-metabolizing enzymes and cytochrome P-450 content. A LOAEL of 3 mg/kg/day was established for this effect. Also, the effect was minimal since the inhibition of enzyme activities was selective and reversible. This dose was divided by an uncertainty factor of 300 (3 for use of a minimal LOAEL, 10 for extrapolation from animals to humans, and 10 for interhuman variability) to yield the calculated MRL of 0.01 mg/kg/day.

No intermediate- or chronic-duration oral MRLs were derived because of a lack of reliable LOAELs and a lack of definite target organs.

Death. Several epidemiology studies have reported increased mortality due to cardiovascular disease in cohorts of workers occupationally exposed to carbon disulfide (Balcarova and Halik 1991; Hernberg et al. 1970, 1973; Swaen et al. 1994; Tolonen et al. 1975, 1979). It is not possible, however, to draw accurate or definitive conclusions from these reports regarding the levels of exposure associated with an effect. This is because of the wide range of exposure levels that workers experience and the difficulty in estimating exact levels from area sampling done at different times and with different methods. Moreover, historical exposure data are lacking in most of the studies, and occupational studies frequently encompass concomitant exposures to other chemicals. However, decreased survival associated with ventricular fibrillation in rats has been noted following oral exposure to carbon disulfide (Hoffman 1987). There are no studies available that address death following dermal exposure in humans or animals.

Systemic Effects. The systemic toxicity of carbon disulfide is manifested primarily as cardiovascular, hepatic, and ocular effects in both humans and animals following inhalation exposure.

Respiratory Effects. Some transient respiratory effects have been noted in humans briefly exposed to unspecified amounts of carbon disulfide (Kamat 1994; Spyker et al. 1982). Limited studies were available that specifically address respiratory function changes in exposed animals. Decreased

respiratory rate was noted in rats exposed to 803 ppm carbon disulfide for 18 hours (Tarkowski and Sobczak 1971).

Cardiovascular Effects. Several studies of occupationally exposed individuals have documented an increased incidence of elevated blood pressure (Egeland et al. 1992; Hernberg et al. 1971; Tolonen et al. 1979; Vanhoorne et al. 1992a). This indicates that an elevation in blood pressure could be one mechanism for the adverse cardiovascular effects of carbon disulfide. Concomitant occupational exposure to hydrogen sulfide may be a confounding factor. At relatively low concentrations (median 1.1 or 4 ppm) cardiovascular effects have not been observed in humans occupationally exposed to carbon disulfide (Chrostek-Maj and Czeczotko 1995a; Drexler et al. 1995b). A transient decrease in blood pressure was reported in rats administered 506 mg/kg carbon disulfide once by gavage (Hoffmann and Klapperstück 1990). ECG changes were seen at 373 and 506 mg/kg, while heart rate decreased at 632 mg/kg. The other studies on animals attest to the adverse effects of carbon disulfide on the cardiovascular system (Antov et al. 1985; Chandra et al. 1972; Wronska-Nofer et al. 1980). These effects included lipid droplet infiltration in the coronary arteries, metabolic and structural changes in the myocardium and the aorta, and myocardial lesions characterized by necrosis, interstitial edema, and cellular infiltrate.

Gastrointestinal Effects. Gastrointestinal symptoms are commonly reported in workers exposed to carbon disulfide (Rubin and Arieff 1945; Vanhoorne et al. 1992b; Vigliani 1954). These symptoms are subjective, however, and cannot be identified with a specific exposure level. No animal studies have been done that address effects on the gastrointestinal system.

Hematological Effects. Fibrolytic activity was decreased in workers exposed to carbon disulfide at 59-169 ppm for 2-8 years (Visconti et al. 1967). Red blood cell and white blood cell counts were not significantly different from preemployment values in workers exposed to carbon disulfide for 5 years (Chrostek-Maj and Czeczotko 1995a).

There are limited animal data concerning hematological effects after inhalation exposure. No hematological effects were seen in rabbits acutely exposed to 1,100 ppm carbon disulfide for 12 days (Briegor 1949). However, exposure to 300 ppm for 48 days resulted in significant increase in pseudo-eosinophils. Significant decreases in erythrocyte counts, total hemoglobin, and hematocrit were noted in mice exposed to 800 ppm for 90 days (Toxigenics 1983c). Animal studies also show an elevation

of serum lipids following carbon disulfide exposure (Wronska-Nofer 1972, 1973; Wronska-Nofer et al. 1980). Vascular arteriosclerotic changes may be a unifying mechanism for the toxic effects caused not only on the cardiovascular system but also in the liver and kidney (Beauchamp et al. 1983).

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans after exposure to carbon disulfide by any route. In rats treated by gavage with carbon disulfide, a decreased response of the ancoccygeus muscle to noradrenaline was observed relative to the vehicle-treated controls (Gandhi and Venkatakrishna-Bhatt 1993). The response of the muscle to noradrenaline was tested *in vitro.*

Hepatic Effects. In humans, inhalation or oral exposure to carbon disulfide causes inhibition of microsomal enzymes and increased liver size (Mack et al. 1974; Vanhoorne et al. 1992b). Similar changes were seen in animals without accompanying histological evidence of liver damage (Magos and Butler 1972; Magos et al. 1973; Tsuyoshi 1959). However, hepatoxicity characterized by hydropic degeneration in parenchymal cells of the centrilobular zone was observed in rats pretreated with phenobarbital to induce the liver mixed-function oxidase system. Effects on lipid metabolism, for example, increased levels of serum cholesterol, total lipids, and triglycerides, have been seen in workers exposed by inhalation to carbon disulfide (Krstev et al. 1992; Stanosz et al 1994b; UK/HSE 1981). Serum cholesterol, phospholipids, and triglyceride levels were significantly elevated in rats following inhalation exposure to carbon disulfide (Wronska-Nofer 1972, 1973). One postulated mechanism involved in the toxicity of carbon disulfide is its suppressive effect on the microsomal hepatic enzyme system (Mack et al. 1974). Many of the hepatic effects of carbon disulfide as well as the interactions of other chemicals with carbon disulfide would then result from the loss of the capability to detoxify other harmful chemicals.

An acute-duration oral MRL was calculated on the basis of decreases in the activities of several hepatic microsomal cytochrome P-450-dependent drug metabolizing enzymes and cytochrome P-450 content in mice after acute exposure to 3 mg/kg/day (Masuda et al. 1986). The calculated acuteduration oral MRL is 0.01 mg/kg/day.

Renal Effects. Although several reviews of the literature on carbon disulfide refer to renal effects in humans, no studies were located that specifically address renal toxicity (Beauchamp et al. 1983; WHO 1986). Rabbits exposed to a wide range of levels of carbon disulfide for 38 weeks showed an

increased incidence of chronic interstitial nephritis (Cohen et al. 1958). Nephropathy was observed in mice exposed via inhalation at 800 ppm for 90 days (Toxigenics 1983c). Because of limited available data, the significance of these findings in animals with regard to adverse effects in humans is not known.

Endocrine Effects. The available data in humans provide conflicting evidence regarding the adverse effects of carbon disulfide exposure on thyroid function (El-Sobkey et al. 1979; Lancrajan et al. 1972; Wagar et al. 1981). Based on decreases in the urinary excretion of products of adrenal/gonadal or adrenal/sympathetic origin, exposure of workers to carbon disulfide may affect adrenal gland function (Cavalleri et al. 1967; Stanosz et al. 1994a). These studies also involved possible exposure to other chemicals and did not identify the precise exposure level.

Dermal Effects. Dermal effects are limited to a report of blisters on the hands of viscose rayon workers presumably due to carbon disulfide exposure. This is borne out by studies in rabbits in which similar blisters could be induced by carbon disulfide exposure (Hueper 1936). This indicates that dermal contact from either occupational exposure or from contaminated soil or water near hazardous waste sites could cause adverse effects.

Ocular Effects. Ophthalmological changes of various types including fundus anomalies, retinal microaneurysms, retinopathy, and burning eyes have been reported in workers occupationally exposed to carbon disulfide (DeLaey et al. 1980; NIOSH 1984a; Raitta et al. 1974, 1975; Rubin and Arieff 1945). These findings suggest that chronic low-level occupational exposure to carbon disulfide or exposure at hazardous waste sites could result in ophthalmological changes.

Body Weight Effects. A 14-day exposure to carbon disulfide at a concentration that resulted in a narcotic-like stupor caused male rats to lose 14% of their body weight (Wilmarth et al. 1993). Inhalation exposure of rats for intermediate durations has been reported to produce a dose-dependent decrease in body weight gain. This effect varied depending upon the sex and the strain of rat used (Clerici and Fechter 1991; Hirata et al. 1992b; Tepe and Zenick 1984; Toxigenics 1983a, 1983b). However, the implications of these findings with regard to adverse effects in humans are unknown.

Other Systemic Effects. Workers occupationally exposed to carbon disulfide exhibited elevated plasma sodium and chloride levels and decreased erythrocyte potassium and calcium levels (Pines

1982). In animals, increased adrenal weight, hyperplasia of adrenal cortex, and mild hemosiderosis of the spleen have been observed (Cohen et al. 1959). However, the value of these studies is limited by confounding factors, lack of dose-response relationships, and limitations in study design.

Immunological and Lymphoreticular Effects. The only study available (Bobnis et al. 1976) reported no immunological component involved in the increase of arteriosclerotic lesions found in carbon disulfide-exposed workers.

Neurological Effects. The primary target organ for carbon disulfide toxicity is the nervous system. In humans, behavioral changes, neurophysiological changes, and neuropathology have been demonstrated (Aaserud et al. 1988, 1990; Chrostek-Maj and Czeczotto 1995b; Chu et al. 1995; Hirata et al. 1992a; Lancranjan 1972; Ruijten et al. 1990, 1993; Sikora et al. 1990). Some studies have noted that these changes were reversed following removal from exposure; other studies have indicated no improvement. One of the major problems has been in establishing levels of exposure associated with the onset of symptoms.

In occupational settings, exposure levels vary daily and over the course of years. Improved technology and an awareness of the harmful effects of carbon disulfide exposure have resulted in a marked decrease in the ambient levels in the workplace from approximately 60 ppm to about 10 ppm. Concomitant exposures to other chemicals remain a problem. Animal data suggest serious neurological effects from carbon disulfide exposure, but most of the studies are single-dose studies using levels of carbon disulfide one to two orders of magnitude larger than those levels seen in occupational settings. Because of difficulties in accurately measuring low concentrations of carbon disulfide in environmental settings and in assessing subtle behavioral or neurological effects, it is hard to draw conclusions about levels or durations of exposure that represent no-effect levels for neurotoxicity in humans. The mechanism of neurotoxicity appears to involve effects on both the axons and the myelin sheaths. In an *in vitro* assay, De Caprio et al. (1992) demonstrated formation of protein-bound isothiocyanate adducts in carbon disulfide-treated peptides and protein. Based on these findings, the study authors proposed that a direct reaction of carbon disulfide with neurofilament lysineamino moieties was a step in the mechanism of neuropathy. Valentine et al. (1993) have shown that the protein in degenerated axons caused by carbon disulfide exposure is greatly crosslinked. Externally, the nerves show swellings paranodally, retraction of myelin from specific nodes, and degeneration of the distal axon (especially long ones). The isothiocyanate adducts formed from

dithiocarbamate adducts cause crosslinking of the nerve proteins, leading to the phenomena observed in electron micrographs.

Valentine et al. (1995) postulated that covalent crosslinking of low molecular weight neurofilament triplet proteins by dithiocarbamates proceeded through liberation of carbon disulfide.

A chronic-duration inhalation MRL was calculated on the basis of reduced motor nerve conduction velocity in humans after occupational exposure to an average of 7.6 ppm carbon disulfide, for individuals working approximately 8 hours a day, 5 days a week, for 12.1 years (the mean exposure period) (Johnson et al. 1983). The calculated chronic-duration inhalation MRL is 0.3 ppm.

Reproductive Effects. Birth defects have been reported in newborns of women workers exposed to carbon disulfide (Bao et al. 1991). However, no definite conclusion can be made because of the inadequacy of available data (lack of exposure analysis and dose-response assessment) since the study was reported only as an abstract. Decreased sperm count and decreased libido in men and menstrual irregularities in women exposed in the workplace have been the most frequently reported effects. In exposed women, possible disruptions of the neurohormonal-endocrine balance necessary for normal ovarian and uterine cycles may lead to amenorrhea, abnormal menstrual cycles, and even sterility (WHO 1979; Zielhius et al. 1984). However, community and workplace studies have not shown a decreased fertility rate, an increase in the time between live births, or an effect on semen quality with carbon disulfide exposure (Hemminki and Niemi 1982; Meyer 1981; NIOSH 1983; Vanhoorne et al. 1974; Zhou et al. 1988). Potential reproductive effects are supported by animal studies, primarily in male rodents, that report decreased sperm count, abnormal coital behavior, and reduced plasma testosterone (Tepe and Zenick 1984; Zenick et al. 1984). These effects are supported by data from intraperitoneal exposures to rats. No effects on the testicular structures were seen at 6.25 mg/kg/day administered for 60 days. Some disorganization of seminiferous tubules was seen at 25 mg/kg/day for 60 days, and a decrease in spermatogenesis was seen at 25 mg/kg/day for 120 days (Gondzik 1971).

Developmental Effects. Although developmental effects have been seen in the offspring of women exposed to carbon disulfide in the workplace (Bao et al. 1991), the data are inadequate to draw any definitive conclusion. The Bao et al. (1991) study was reported as an abstract, and there was a lack of exposure analysis and dose-response assessment. There are reports of congenital malformations in the offspring of rats exposed via inhalation (Tabacova and Balabaeva 1980a), but the general finding in

the animal studies of developmental effects is one of increased embryotoxicity (Lehotzky et al. 1985; Yaroslavskii 1969). In a preliminary study reported only in abstract form, increases in early resorptions and decreases in viable fetuses were observed in New Zealand white rabbits exposed by inhalation to 600 or 1,200 ppm (Gerhart et al. 1991). No effects were noted at 300 ppm. Pharmacokinetic studies indicate that carbon disulfide and its metabolites pass the placenta at all stages of gestation and localize in the recognized target organs for this chemical (brain, blood, liver, and eyes) (Danielsson et al. 1984), but the levels at which these exposures could produce effects in humans is not identifiable at this time.

Genotoxic Effects. There are some data on *in vitro* genotoxicity of carbon disulfide. Haworth et al. (1983) evaluated carbon disulfide at five doses both with and without S9 activation in the *Salmonella*/microsome assay. All results were negative. Other studies in *Salmonella typhimurium* and *Escherichia coli*, both with and without activation, were also negative (Donner et al. 1981; Hedenstedt et al. 1979). *Salmonella* strain TA98 was used in a host-mediated assay investigating the ability of carbon disulfide to induce reverse mutations. Carbon disulfide was not considered to be an active mutagen in this test; however, the results are equivocal in this regard (NIOSH 1980). A response curve suggestive of carbon disulfide-induced unscheduled deoxyribonucleic acid (DNA) synthesis was observed in human WI-38 cells, but the results failed to meet the criteria indicative of a positive response (NIOSH 1980). Carbon disulfide was not considered to be an a mutagen in this test. Studies in lymphocytes (Garry et al. 1990) demonstrated a requirement for microsomal activation in the induction of dose-related ($p \leq 0.05$) increases in sister chromatid exchanges. This finding is consistent with past work on the cytochrome P-450 monooxygenase system that suggests a requirement for microsomal activation to form a highly reactive metabolite. Carbon disulfide was reported to inhibit the mutagenic activity of 1,2-dimethylhydrazine and azoxymethane in a host-mediated assay (Moriya et al. 1979). In this report, the authors suggested that this effect was due to carbon disulfide's prevention of *in vivo* oxidation of azomethane to azoxymethane. This would be consistent with the known suppressive effects of this chemical on the cytochrome P-450 system. Results of carbon disulfide on the *Drosophila* sex-linked recessive lethal test were also negative (Donner et al. 1981; NIOSH 1980). Carbon disulfide failed to produce significant chromosomal aberrations in the bone marrow of rats inhaling carbon disulfide for either acute or intermediate periods (NIOSH 1980). There are no mutagenicity data on humans exposed occupationally to carbon disulfide, so at this time it is difficult to predict effects of carbon disulfide at the DNA level.

Cancer. There are no definitive data in humans or animals that indicate a carcinogenic potential for carbon disulfide. In the absence of positive genotoxic data, increased cancer risk does not appear to be an effect of exposure to carbon disulfide.

2.6 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s), or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to carbon disulfide are discussed in Section 2.6.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not

be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by carbon disulfide are discussed in Section 2.6.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.8, "Populations That Are Unusually Susceptible."

2.6.1 Biomarkers Used to Identify or Quantify Exposure to Carbon Disulfide

Levels of carbon disulfide detected in exhaled breath, blood, urine, and milk as well as various metabolite concentrations in the urine of exposed individuals have been studied as biomarkers of carbon disulfide exposure.

Because of its solubility in lipids and affinity for proteins, environmental carbon disulfide is quickly absorbed in the blood and other body tissues through inhalation or cutaneous absorption. The absorbed carbon disulfide is then eliminated through the lungs unchanged as carbonyl sulfide or as carbon dioxide, or it is converted to water-soluble metabolites, which are excreted by the kidneys. Metabolites of carbon disulfide found in the urine are often utilized as biomarkers of exposure. Sulfates, for example, are nonspecific products of the metabolism of carbon disulfide and many other compounds. Some investigators have observed an increase in total urinary sulfate levels (specifically inorganic sulfates) in humans after exposure to 22 ppm of carbon disulfide vapor for 1.5 hours (McKee et al. 1943) suggesting that sulfate levels may be useful as a nonspecific biomarker of exposure, perhaps in combination with other markers. However, other researchers have made conflicting observations. More recently, certain specific dithiocarbamates have been used in more specific and reliable tests (discussed below) of carbon disulfide exposure.

Carbon disulfide is also found in the saliva and sweat of exposed individuals in small quantities. These measurements have not been shown to quantitatively correlate with carbon disulfide exposure. The concentration of carbon disulfide in the feces is very low and therefore has also not been routinely used as a biological marker of exposure (Djuric 1967).

Higher cholesterol levels (correlated with exposure levels), higher blood creatinine levels, marked disturbances of the hepatic cytochrome P-450 content and of the associated microsomal monooxygenase system, as well as the inhibition of succinic-oxidase enzyme activity, may also be considered as nonspecific biomarkers of carbon disulfide exposure. More research, however, needs to be done in order to determine whether a direct correlation exists between these parameters and carbon disulfide exposure.

The following paragraphs describe reasonably specific biomarkers for carbon disulfide exposure in humans that correlate with exposure levels to varying degrees.

Carbon disulfide levels eliminated by exhalation may yield some information about recent, short-term exposure; however, the breath test has not been widely used for monitoring occupational exposure because of several confounding factors. The first phase of carbon disulfide elimination by breath is very fast. Pollutant concentrations in the breath, therefore, may reflect variable uptake during the day but may not be a good reflection of the longer term uptake and exposure values for an entire working day. Results from breath tests for carbon disulfide exposure are further confounded by the fact that absorption and elimination rates of carbon disulfide by the lungs are variable among different individuals and within the same individual because of differences in activity and metabolic rates. Chronically exposed persons, for example, have a lower retention rate than those exposed for the first time (Campbell et al. 1985; Djuric 1967).

Because of these confounding factors, results of breath tests for carbon disulfide exposure do not correlate well with its environmental levels. However, one might be able to detect carbon disulfide exposure more reliably if measurements of carbon disulfide were made during a second slower elimination phase and if a more sensitive detection technique were used. A quadrupole mass spectrometer makes the measurement of carbon disulfide in exhaled breath much more sensitive, detecting exposure levels as low as 1 ppm. In an investigation by Campbell et al. (1985), the shortterm elimination of carbon disulfide was studied measuring uptake that had taken place l-2 hours before the test. Carbon disulfide levels in the breath were found to fluctuate, but the value of next-day tests measuring the slower elimination phase of carbon disulfide by the breath should be explored. Nevertheless, the use of exhaled carbon disulfide remains an equivocal biomarker of exposure.

Blood carbon disulfide levels may also serve as a biomarker of exposure. Data from blood tests, however, have not shown consistent correlation with carbon disulfide exposure levels in past studies. Blood carbon disulfide concentration after exposure may be a poor indicator of actual exposure because of rapid clearance from this tissue (Beauchamp et al. 1983; WHO 1979). A confounding factor in using blood carbon disulfide concentration as a biomarker of exposure is the fact that two different species of carbon disulfide exist in the blood. “Free” carbon disulfide is unbound carbon disulfide that is still dissolved in blood plasma; “bound” or “acid-labile” carbon disulfide refers to an appreciable amount of carbon disulfide dissolved in plasma lipids or bound to proteins in the blood. The equilibrium between free and acid-labile carbon disulfide in blood varies inter- and intraindividually, rendering blood carbon disulfide measurements less reliable for calculating exposure dose. Moreover, bound carbon disulfide’s rate of release into other body systems may vary also. A head-space gas chromatography detection technique used to measure acid-labile carbon disulfide may bear investigation. Carbon disulfide levels are usually measured in acid-treated whole blood; however, it is suspected that the greater percentage of carbon disulfide in the blood is bound to blood cell membranes because of its affinity for lipids and proteins (Campbell et al. 1985). This means that the lack of correlation of blood plasma levels of carbon disulfide with exposure data may be due to a significant portion of the carbon disulfide not being measured at all as it is not in the plasma.

Some investigators have measured levels of carbon disulfide in urine as a biomarker of exposure, but this is probably optimal only for measuring high exposure levels (Beauchamp et al. 1983). Good correlation between urinary carbon disulfide levels and work exposure was not found in any studies. The measurements of excreted or “free” carbon disulfide may have been confounded by the presence of various thiometabolites or “bound” species. Moreover, the volatility of carbon disulfide and individual variations in urinary flow rate and metabolism may have confounded results in these studies (Djuric 1967).

The concentration of crosslinked red blood cell spectrin has been suggested as a marker of nerve protein crosslinking damage that leads to slower conduction velocities and abnormal nerves due to protein adduct formation initially with dithiocarbamates which decompose to form isothiocyanate adducts (Valentine et al. 1993). These latter adducts can then cause the actual crosslinking of both spectrin and nerve protein. As new red blood cells must be made to replace the damaged spectrin, the crosslinking of this protein may serve as a longer term biomarker of carbon disulfide exposure.

The iodine-azide test measures possible carbon disulfide exposure, based on the decolorization of iodine in the presence of urinary carbon disulfide thiometabolites. The iodine-azide test is a means for determining longer term carbon disulfide exposure at air concentrations of 50 mg/m^3 (around 16-20 ppm) and above (Baselt 1980; Beauchamp et al. 1983; Campbell et al. 1985; Lieben 1974; WHO 1986). However, it is not a very sensitive test for exposure at lower concentrations of concern to OSHA. The iodine-azide test measures the catalysis of the reaction between iodine and sodium azide by the following metabolites of carbon disulfide acting as biomarkers of exposure: thiourea, which is the main urinary thiometabolite involved in this test, 2-thio-5-thiazolidinone, and a third unidentified metabolite. The latter two chemicals are present in very small quantities and are not as important in catalyzing the iodine-azide reaction (Beauchamp et al. 1983). However, even though exposure can be detected, the utility of such measurements is limited by the lack of the correlation between exposure and urinary carbon disulfide described in the previous paragraph; that is, level or duration of exposure cannot be quantified.

Measuring the total concentration of urinary thio compounds (including glutathione conjugates, mercapturic acids, and other sulfur-containing carbon disulfide metabolites) can serve as a good marker of exposure. The level of total thio compounds correlates with carbon disulfide exposure levels and is a more sensitive biomarker of exposure than the iodine-azide test (Beauchamp et al. 1983; Van Doorn et al. 1981a). This biomarker detects an exposure to 6 ppm carbon disulfide for 8 hours (Beauchamp et al. 1983). These compounds are not absolutely specific for carbon disulfide exposure.

TTCA is used as an indicator to assess the degree of occupational exposure to carbon disulfide (Thienpont et al. 1990). Although the detection limit was estimated at 0.05 mg of TTCA/g of urinary creatinine, the study did not provide data on carbon disulfide exposure levels.

In a rayon production factory, exposure to carbon disulfide was measured by personal air sampling and the excretion of TTCA in urine (Meuling et al. 1990). Based on the personal air-sampling, the TWA exposure level for carbon disulfide was 12.6 mg/m^3 (4 ppm). The study authors established a calculated biological limit value of 0.77 mg TTCA/g creatinine (0.57 mmol/mol creatinine) to correspond with 95% confidence, to a TWA air concentration lower than the threshold limit value (TLV) of 30 mg/m^3.

Measuring levels of one particular urinary thiometabolite of carbon disulfide may serve as an even more sensitive and specific biomarker of exposure. TTCA, which is a product of glutathione conjugation, is quantitatively related to carbon disulfide uptake (Beauchamp et al. 1983; Campbell et al. 1985; Drexler et al. 1994). Carbon disulfide exposure levels of 2.5 ppm and above are detected by this analytical method. Carbon disulfide exposure correlates well with urinary TTCA concentrations, especially once the metabolite levels are normalized to urinary creatinine content. It appears that TTCA levels may be used as a sensitive monitor of longer term exposure in workers (Beauchamp et al. 1983; Campbell et al. 1985; Drexler et al. 1994). One limitation of urinary TTCA levels is that this compound has been detected at low concentrations (range, 0.005-0.15 mg/g creatinine) in persons not exposed to carbon disulfide (Lee et al. 1995). The source of this TTCA is thought to be from dietary intake, especially the consumption of brassica vegetables (e.g., cabbage) (Simon et al. 1994). Therefore, in persons who eat large amounts of these vegetables, measurements of urinary TTCA may overestimate carbon disulfide exposure. Baseline sampling is therefore necessary to correct for nonworkplace exposure sources.

Biological monitoring for carbon disulfide exposure was performed using the iodine-azide test and TTCA test in urinalysis of workers with high exposure to carbon disulfide (36-46 ppm) (van Poucke et al. 1990). Based on the findings of the study, the specificity and the sensitivity were low for the iodine-azide test and high for the TTCA test. ACGIH (1986, 1994) has recommended a biological exposure index of end-of-shift urinary TTCA of 5 mg/g creatinine.

Therefore, it appears that because of the limitations in the methodology for measuring carbon disulfide in blood, breath, and urine of exposed individuals, direct measurement of this compound is not the most sensitive test for determining the extent of exposure (Beauchamp et al. 1983; Campbell et al. 1985; Djuric 1967; McKee et al. 1943; WHO 1979). For the present, the biomarker that correlates best with exposure is measurement of metabolites in the urine. The iodine-azide and TTCA tests can be conducted to measure urinary levels of carbon disulfide metabolites as they have been shown to correlate with exposure (Baselt 1980; Beauchamp et al. 1983; Campbell et al. 1985; Lieben 1974; WHO 1986), with the TTCA test being more sensitive and specific than the iodine-azide test.

For more information concerning absorption, distribution, metabolism, and excretion of carbon disulfide, refer to Section 2.3.

2.6.2 Biomarkers Used to Characterize Effects Caused by Carbon Disulfide

The battery of biomarkers discussed here may be used as indicators of probable carbon disulfide exposure. However, the physiological effects of carbon disulfide poisoning are numerous and range from mild to severe. Their utilization as biomarkers of effect are confounded by their occurrence in response to other epidemiological, nutritional, and environmental factors. Their significance as biomarkers is further reduced by the fact that these effects occur with great variance in the cohort exposed population.

The following are proposed as likely biomarkers of effect for carbon disulfide; however, more information about their possible correlation with actual carbon disulfide exposure and their reliability and consistency is necessary before they can be utilized to indicate level or duration of exposure or predict potential health effects.

Several neurological parameters may be useful as more specific biomarkers of polyneuropathy from carbon disulfide exposure. CT-scans, magnetic resonance imaging, and pneumoencephalography (PEG) may indicate early cerebral/cerebellar atrophy in humans (Beauchamp et al. 1983; Peters et al. 1988). EMGs have detected signs of neurogenic lesions in humans, and changes in brain EEG patterns in animals have accompanied carbon disulfide-induced central nervous system toxicity. Moreover, neurophysiological methods may be utilized to detect decreasing nerve conduction velocity, which is a biomarker of peripheral nervous system effects (WHO 1981).

Changes in lipid metabolism are the most obvious biomarkers of carbon disulfide's vasculopathic effects. Hypercholesterolemia (Toyama and Sakurai 1967) and high β-lipoproteins in the blood (Prerovska and Drdkova 1967) have been observed by investigators following long-term occupational carbon disulfide exposure. Elevated blood lipid concentrations following long-term carbon disulfide exposure in humans may be an appropriate indicator of ensuing arteriosclerosis, clinical vasculopathy, and increased risk of cardiovascular disease (El-Sobkey et al. 1979). However, the accuracy and reliability of this parameter as a potential biomarker of exposure for carbon disulfide are in question since many things can cause changes in lipid metabolism.

More specific blood lipid parameters, however, may prove to be useful in the future. Changes have been observed in lipid metabolism when a cytochemical enzymological examination of leukocytes and

platelets was carried out for over 600 exposed workers (Micu et al. 1985). Researchers found high levels of lymphocytic lipids and low levels of granulocytic lipids. Another investigator found elevated serum cholesterol and fatty acids and low cholesterol ester levels in an 11-week study of dogs. However, only the experimental animal group fed a high-fat diet showed altered lipid metabolism. The exposed groups on normal and high-carbohydrate diets had normal serum lipid content (Lewey et al. 1941).

In exposed women, possible disruptions of the neurohormonal-endocrine balance necessary for normal ovarian and uterine cycles may lead to amenorrhea, abnormal menstrual cycles, spontaneous abortions, and even sterility (WHO 1979; Zielhuis et al. 1984). Serum thyroxine levels, which decrease following carbon disulfide exposure, have also been suggested as a biomarker (Cavalleri 1975).

Higher plasma creatinine levels were observed among workers exposed to 4-18 ppm of ambient carbon disulfide. Creatinine level in plasma may be utilized as a nonspecific biomarker of short-term renal dysfunction (Hernberg et al. 1971). Another such biomarker of renal effects may be the blood sugar level. Higher than normal blood sugar levels in response to carbon disulfide exposure were observed in a chronic-duration human study (Hernberg et al. 1971) and an intermediate-duration dog study (Lewey et al. 1941).

In studying the effects of carbon disulfide exposure on enzyme systems of carbohydrate metabolism, McKee et al. (1943) observed that the succinic-oxidase system was inhibited. They noted a 10% decrease in the activity of this system. Carbohydrate metabolism is crucial in proper neural function; thus, succinic-oxidase activity may serve as an appropriate biomarker of nervous system effects (McKee et al. 1943).

In conclusion, the following summarizes possible correlative biological markers of early carbon disulfide poisoning: (1) electromyographical indications of neural lesions; (2) decreased neuromuscular conduction velocity; (3) abnormal lipid metabolism as indicated by hypercholesterolemia; (4) decreased steroid hormone levels (Lieben 1974); (5) low urinary thiamine levels; (6) high plasma creatinine levels; and (7) lower succinic-oxidase enzyme activity (Beauchamp et al. 1983; Hernberg et al. 1971; Lewey et al. 1941; WHO 1981). In addition, covalent crosslinking to erythrocyte spectrin may find application as a biomarker (Valentine et al. 1993). All of these are early indicators of central and peripheral nervous system, cardiovascular, endocrine, and reproductive

toxicity. Also, these biological markers are not specific for carbon disulfide. One or a combination of these markers may prove to be a useful biomarker for carbon disulfide effects. See Section 2.2 for other effects caused by carbon disulfide.

2.7 INTERACTIONS WITH OTHER SUBSTANCES

Many of the chemical interactions with carbon disulfide appear to be related to loss of microsomal cytochrome P-450. Carbon disulfide suppresses the hepatic cytochrome P-450 microsomal enzyme system. Elimination of phenazone, a drug often used in the study of hepatic microsomal enzyme activity, is significantly and reversibly inhibited in rabbits exposed to 193 ppm carbon disulfide for 5 hours a day, 6 days a week, for 6 months (Orzechowska et al. 1984). It has been proposed that the active sulfur atoms released following carbon disulfide metabolism suppress the cytochrome P-450 enzymes, thus inhibiting detoxification of other drugs or chemicals.

The influence of carbon disulfide exposure on the cardiovascular actions of adrenaline and noradrenaline was examined in urethane-anesthetized rats. Electrocardiographic change (T-wave elevation) consistent with slight myocardial ischemia was noted in anesthetized rats administered carbon disulfide (253 mg/kg/day) for 4 weeks and challenged with adrenaline or noradrenaline (Hoffmann and Muller 1990; Klapperstück et al. 1991). Carbon disulfide-exposed rats were more prone to ventricular arrhythmias (extrasystoles) and a prolongation of the PR interval than nontreated animals. Thus, the hypertensive adrenergic effects of these drugs appear to be enhanced in carbon disulfide-exposed rats. It was further shown by Klapperstück et al. (1991) that lactic dehydrogenase M isozyme activity was increased under these condition, while total lactate dehydrogenase activity was not, a change that has been associated with adaptation to anaerobic metabolism. Based on these findings, the study authors postulated that carbon disulfide cardiotoxicity may be mediated by disruption of the energy supply. Administration of up to 506 mg/kg carbon disulfide by gavage to urethane-anesthetized Wistar rats did not cause changes in the development of cardiac arrhythmias induced either by ischemia (coronary ligation) or by aconitine administration (Hoffmann and Klapperstuck 1990).

The combined effect of carbon disulfide exposure and ethyl alcohol has been examined to determine if carbon disulfide exposure results in the Antabuse syndrome, an intolerance to alcohol. The metabolism of Antabuse, disulfuram, or tetraethylthiuram disulfide (TETD) produces carbon disulfide

and diethylamine. The metabolites of Antabuse inhibit the enzymes necessary to metabolize ethyl alcohol (aldehyde dehydrogenase and catalase), which results in the Antabuse syndrome due to a buildup of aldehyde. Symptoms include a sensation of heat, a fall in blood pressure, nausea, and in extreme cases circulatory collapse (Djuric 1971). Research by Freundt et al. (1976) on rats and humans of the combined effects of carbon disulfide exposure and ethanol ingestion indicate that, at low (20 ppm) and medium (400 ppm) levels of carbon disulfide exposure and blood alcohol levels of approximately 0.75%, there is a carbon disulfrde inhibition of aldehyde dehydrogenase with an increase in acetaldehyde concentrations in the blood. However, these increased acetaldehyde concentrations were not considered great enough to indicate the Antabuse syndrome. The study authors asserted that the Antabuse syndrome is not likely to occur in subjects who have blood alcohol levels of up to 0.8% and are exposed to 10 ppm carbon disulfide.

The possible role of the ethanol-inducible isozyme of cytochrome P-450 in the metabolism of carbon disulfide has been examined (Snyderwine et al. 1988). Rats were administered various alcohols (methanol, ethanol, isopropanol, and isobutanol) by gavage. Eighteen hours after alcohol administration, rats were administered carbon disulfide intraperitoneally at doses of 1, 100, or 625 mg/kg. The results showed that pretreatment of rats with these alcohols enhances the metabolism of carbon disulfide by increasing the ethanol-inducible isoform of cytochrome P-450 with isopropanol being most potent. Furthermore, the study authors indicate that alcohol induction of P-450-dependent carbon disulfide metabolism per se is not sufficient to result in carbon disulfide-induced hepatic damage although it does lead to the loss of specific cytochrome P-450 function.

The chronic effect of carbon disulfide and ethanol was examined by Opacka et al. (1984). Rats were exposed to 257 ppm of carbon disulfide for 5 hours a day, 6 days a week, for 11 months, and 10% ethanol (in water *ad libitum* for the last 3 months, control water *ad libitum*). Control rats were exposed to filtered air. The behavior, memory, and learning ability of the ethanol-fed rats were adversely affected compared to controls. Additional studies indicate biochemical alterations in the central nervous system and increased β-glucuronidase activity; ultrastructural studies show degeneration in the peripheral nervous system, particularly in the myelin sheath. These authors reported that the effects from combined exposures are greater than those from each substance alone. Wronska-Nofer et al. (1986) investigated the hepatotoxicity of combined ethanol and carbon disulfide in the rat. Rats were chronically exposed to 482 ppm (1.5 g/m^3) of carbon disulfide 5 hours a day, 5 days a week, for 5 months and given a 10% ethanol solution as their sole source of fluids. Ethanol

increased the hepatotoxicity of carbon disulfide by potentiating the effects (further depressing the P-450 levels) of carbon disulfide on the activity of the microsomal cytochrome P-450 monooxygenases of the liver and on the hepatic endoplasmic reticulum (development of giant mitochondria and degranulation of the rough endoplasmic reticulum).

An occupational epidemiological study of grain workers indicates that there are neurotoxic effects from exposure to the 80/20 carbon tetrachloride/carbon disulfide fumigant. Most of the effects (dysfunction of the peripheral axons, auditory nerve, optic nerve, and extrapyramidal system, as well as altered behavior and cognition) also appear in viscose rayon workers exposed to carbon disulfide. However, a combined effect with carbon tetrachloride could not be ruled out. EPA banned the use of 80/20 carbon tetrachloride/carbon disulfide fumigants after June of 1986 because of the potential for combined and synergistic toxic effects on the nervous system (EPA 1989a). In sheep, combined treatment with carbon tetrachloride and carbon disulfide, whether given simultaneously or separated by 6 hours (carbon disulfide then carbon tetrachloride), is effective in controlling liver fluke (Seawright et al. 1972). Carbon tetrachloride can be hepatotoxic in sheep; when it is administered with carbon disulfide, however, the toxicity is significantly reduced. The mechanism by which carbon disulfide protects against carbon tetrachloride toxicity in rats was investigated by Seawright et al. (1980). Carbon tetrachloride requires microsomal metabolism in the liver to exert a toxic effect. Carbon disulfide is effective in decreasing microsomal metabolic activity through the loss of cytochrome P-450 and therefore decreases the microsomal metabolism of carbon tetrachloride.

Carbon disulfide potentiates the toxic effect of amphetamines in rats; this effect increases with duration of exposure to carbon disulfide (Caroldi et al. 1987). Freundt et al. (1974b) have found that the effect of sodium phenobarbital pretreatment on rat liver fat accumulation following exposure to carbon disulfide is dependent on the dose and method of ingestion of carbon disulfide. Fat accumulation is significant if the phenobarbital-treated rat is exposed orally to a very high dose of carbon disulfide (1,263 mg/kg body weight); however, inhalation of 20-200 ppm of carbon disulfide (8 hours for 2-7 days) in a phenobarbital-treated rat did not produce fat accumulation in the liver. Extrapolation to human beings is difficult, but the study authors asserted that the sensitivity of hepatic MFOs to carbon disulfide is similar qualitatively and quantitatively in rats and humans. They suggest that it is not hazardous for individuals without hepatic disease to take barbiturate-containing drugs and simultaneously work in 20-200 ppm carbon disulfide (Freundt et al. 1974b).

Carbon disulfide interacts with several organophosphorus compounds including the insecticides malathion and parathion. Metabolism of malathion and parathion requires cytochrome P-450 and is thus inhibited by carbon disulfide (Dalvi and Howell 1978). It is important to note that carbon disulfide would potentiate the toxic effect of compounds that require cytochrome P-450 microsomal metabolism for detoxification.

Oral administration of diethylthiocarbamate and carbon disulfide (separately) protects mice against chloroform-induced kidney injury (Masuda and Nakayma 1983a). The mechanism for protection is postulated to be the inhibition of the kidney microsomal mono-oxygenase system by the thiono-sulfur groups of diethylthiocarbamate and carbon disulfide because chloroform requires metabolic activation in the kidney to induce nephrotoxicity. Carbon disulfide also protects mice from 1,2-dimethyl-hydrazine-induced neoplasia of the large intestine (Wattenberg and Fiala 1978). 1,2-Dimethyl-hydrazine requires several steps of metabolic activation, including the *N*-oxidation of azomethane to azoxymethane. This step is inhibited by the loss of microsomal MFO activity caused by carbon disulfide.

In a well-conducted study by Antov et al. (1985), rats were administered carbon disulfide by inhalation for l-6 months with or without an arteriogenic diet (consisting of cholesterol, choleic acid, and vitamin D2), which was used to develop the sclerotic process. Concentrations of 16, 32, and 64 ppm carbon disulfide produced significant changes in the myocardium in a dose-response relationship. The administration of the atherogenic diet potentiated the cardiotoxicity and enhanced the sclerotic process. Although changes in enzyme activity (statistical significance not reported) were noted, no substantial structural changes occurred in the heart muscle or aorta at the lowest dose of carbon disulfide tested (3.2 ppm) without the atherogenic diet. Histological changes characterized by vacuolar dystrophy and interstitial fibrosis occurred at 16 ppm carbon disulfide and became more advanced at higher concentrations (64 ppm). The study authors concluded that carbon disulfide has an arteriogenic effect.

Carbon disulfide was reported to inhibit the mutagenic activity of 1,2-dimethylhydrazine and azoxymethane in a host-mediated assay (7-week-old ICR mice) (Moriya et al. 1979). These authors suggested that this effect is due to carbon disulfide’s prevention of in *vivo* oxidation of azomethane to azoxymethane.

2.8 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to carbon disulfide than will most persons exposed to the same level of carbon disulfide in the environment. Reasons include genetic make-up, developmental stage, age, health and nutritional status (including dietary habits that may increase susceptibility, such as inconsistent diets or nutritional deficiencies), and substance exposure history (including smoking). These parameters may result in decreased function of the detoxification and excretory processes (mainly hepatic, renal, and respiratory) or the pre-existing compromised function of target organs (including effects on clearance rates and any resulting end-product metabolites). For these reasons we expect the elderly with declining organ function and the youngest of the population with immature and developing organs will generally be more vulnerable to toxic substances than healthy adults. Populations who are at greater risk due to their unusually high exposure are discussed in Section 5.6, “Populations With Potentially High Exposure.”

There are studies that have investigated particular metabolic traits that may result in hypersusceptibility to carbon disulfide (Djuric et al. 1973; Stokinger and Scheel 1973). The study conducted by Djuric et al. (1973) reported on 72 workers who had been divided into three groups: 18 exposed to carbon disulfide at levels below the industrial air limit of 20 ppm (60 mg/m^3) (controls), 21 who had been exposed to levels higher than 20 ppm but had shown no signs or symptoms of carbon disulfide intoxication (resistant), and 33 who had polyneuritis or other signs of overexposure and had been removed from exposure (susceptibles). All individuals were administered an oral dose of 0.5 g of disulfiram (Antabuse), a compound that produces carbon disulfide when metabolized. It was assumed that carbon disulfide and disulfiram are metabolized by the same or similar enzyme system, and determination of diethyl dithiocarbamates (DDC) in urine after disulfiram administration was used to evaluate the rate at which sulfur compounds are metabolized. The excretion of DDC was significantly lowest in the susceptible group (49.70 μg/mg creatinine) when compared to both the control (160.05 μg/mg creatinine) and resistant (90.04 μg/mg creatinine) groups. These results led to the suggestion that the reduced ability of the symptomatic workers to metabolize this compound would lead to hypersusceptibility to carbon disulfide and would thus be associated with the clinical signs observed in that group. No supporting data have been located, however.

The study authors (Djuric et al. 1973) suggested that carbon disulfide exposure causes a decrease in excretion of DDC, especially in once-poisoned workers; thus carbon disulfide exposure produced a

disturbance in the metabolism of sulfur compounds. They also suggested that in the susceptible worker group this decreased metabolic conversion appeared to persist even 5-10 years after exposure, and thus carbon disulfide exposure may have led to an irreversible metabolic disturbance. These authors did not speculate on the mechanism of actual metabolic inhibition, nor did they propose any genetic hypothesis. One study limitation included the problematic issue of whether it is possible to establish that a prior hypersusceptibility existed in testing workers who had been exposed for long periods of time and whose differences in metabolism may have related to the circumstances of their exposure rather than to a previous susceptibility.

Because it appears that one common mechanism of the cerebral, cardiovascular, and hepatic effects may be an acceleration of the arteriosclerotic process (see Section 2.4), individuals at risk for arteriosclerosis or those with early arteriosclerosis would probably be at increased risk for health effects following exposure to carbon disulfide (NIOSH 1978). The mechanism for carbon disulfide acceleration of arteriosclerotic plaque formation involves direct injury to the vessel endothelium and changes in lipid metabolism.

Three other groups are recognized as being unusually susceptible to carbon disulfide: alcoholics (including those treated with Antabuse), those with neuropsychic disorders, and those with vitamin B_6 deficiency (Djuric et al. 1973; Lefaux 1968; Peters et al. 1982). Carbon disulfide reduces the levels of vitamin B_6, which in turn upsets carbohydrate metabolism, particularly the cerebral carbohydrates (Lefaux 1968).

2.9 METHODS FOR REDUCING TOXIC EFFECTS

This section describes clinical practice and research concerning methods for reducing toxic effects of exposure to carbon disulfide. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to carbon disulfide. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

2.9.1 Reducing Peak Absorption Following Exposure

There are no specific methods available to reduce the absorption of carbon disulfide following exposure. Activated charcoal (Stutz and Janusz 1988) and gastric lavage or induced emesis using a saturated sodium bicarbonate solution to decrease gastric acidity and to preclude hydrogen sulfide formation (Dreisbach and Robertson 1987) have been suggested for treatment of carbon disulfide ingestion. However, the use of emetics is controversial (Bronstein and Currance 1988; Stutz and Janusz 1988). If contamination occurs via the skin, thorough washing with soap and water has been suggested. For eye contamination, flushing the eyes with copious amounts of water is the recommended treatment (Stutz and Janusz 1988).

Urea (0.5-1.5 g/kg) administered intravenously has been recommended to inactivate free carbon disulfide in the blood; intravenous administration of large doses of vitamin B_6 has also been recommended (HSDB 1995).

2.9.2 Reducing Body Burden

Unmetabolized carbon disulfide is exhaled unchanged in expired air (McKee et al. 1943), whereas metabolites are excreted primarily in the urine (Soucek 1957). Therefore, increasing ventilation and urinary output may be potential methods to facilitate mitigation of the effects of carbon disulfide. However, such methods have not been attempted with regard to carbon disulfide within a clinical setting. Removing the patient from the contaminated area followed by supportive care is the suggested basic form of treatment (Bronstein and Currance 1988; Dreisbach and Robertson 1987; Ellenhorn and Barceloux 1988; Haddad and Winchester 1990; Morgan 1982; Stutz and Janusz 1988).

Carbon disulfide is metabolized by cytochrome P-450 to an unstable oxygen intermediate which may either spontaneously degrade to atomic sulfur and carbonyl sulfide or hydrolyze to form atomic sulfur and monothiocarbamate (Beauchamp et al. 1983). Although phenobarbital pretreatment of rats can enhance the metabolism of carbon disulfide (Dalvi and Neal 1978), it can also enhance the hepatic toxicity of carbon disulfide. Another metabolic pathway for carbon disulfide is its conjugation with glutathione to form 2-thiothiazolidine-4-carboxylic acid and 2-oxothiazolidine-4-carboxylic acid,which are excreted in the urine (Van Doorn et al. 198la, 1981b). Although the extent to which glutathione

conjugation plays a role in the metabolism of carbon disulfide is unclear, it is conceivable that administration of cysteine may be utilized in the mitigation of the effects of carbon disulfide.

2.9.3 Interfering with the Mechanism of Action for Toxic Effects

There are no specific methods available that provide information on interfering with the mechanism of action for toxic effects of carbon disulfide. However, decreases in carbon disulfide toxicity may be brought about by other chemical substances. For example, the effects of carbon disulfide on the nervous system (the primary target of exposure to carbon disulfide) appear to be influenced by the mineral content of the diet. Rabbits given diet supplements of copper and zinc were exposed to high levels of carbon disulfide (1,100 ppm) and did not show the usual signs of exposure-weight loss, serum lipoprotein and total cholesterol increase, adrenal hypertrophy, and pathological changes in the brain and spinal cord-that appeared in controls on a normal salt diet (ACGIH 1986). A proposed biochemical basis for the antagonist effect of minerals is that carbon disulfide metabolites-dithiocarbamates- are metal chelating agents. The metal complex is more water soluble than the dithiocarbamate alone and thus is excreted faster than the dithiocarbamate. Additional minerals hasten the loss of the compound that causes the effects.

The formation of reactive intermediates resulting from the metabolism of carbon disulfide by the hepatic MFO system may be involved in the hepatoxicity of this chemical. Pretreatment of rats with phenobarbital, an inducer of MFO activity, can enhance carbon disulfide-induced hepatotoxicity (Bus 1985). Inhibition of the MFO system might potentially reduce carbon disulfide-mediated hepatoxicity. In support of this possibility, Bond and De Matteis (1969) reported that pretreatment of rats with SKF-525A, an inhibitor of cytochrome P-450-mediated metabolism, reduced the liver damage from carbon disulfide in phenobarbital-pretreated animals.

Two possible mechanisms for the neurotoxicity of carbon disulfide have been suggested. One mechanism involves the formation of dithiocarbamates. The inhibitory effect of carbon disulfide on the activity of the copper-requiring enzyme dopamine-β-hydroxylase was attributed to the formation of dithiocarbamates, which can complex copper (McKenna and DiStefano 1977b). Interference with the formation of this metabolite may be a potential strategy, albeit untested, to reduce neurotoxicity from carbon disulfide poisoning. An alternative mechanism postulated to explain the neurotoxic effect of carbon disulfide is the formation of a dithiocarbamate derivative, a form of vitamin B_6, of

pyridoxamine, with carbon disulfide (Vasak and Kopecky 1967). Since transaminases and amine oxidases require the pyridoxamine phosphate form of vitamin B_6 as a cofactor, it was further postulated that these enzymes would be inhibited in carbon disulfide poisoning. Although data are limited, supplementation of the diet with vitamin B_6 has been demonstrated to delay some of the neurotoxic effects of carbon disulfide (Teisinger 1974).

2.10 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of carbon disulfide is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of carbon disulfide.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

2.10.1 Existing Information on Health Effects of Carbon Disulfide

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to carbon disulfide are summarized in Figure 2-4. The purpose of this figure is to illustrate the existing information concerning the health effects of carbon disulfide. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a “data need.” A data need, as defined in ATSDR’s Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

FIGURE 2-4. Existing Information on Health Effects of Carbon Disulfide

	Death	Acute	Intermediate	Chronic	Immunological/Lymphoreticular	Neurological	Reproductive	Developmental	Genotoxic	Cancer
	SYSTEMIC									
Inhalation	●	●		●	●	●	●	●		●
Oral	●									
Dermal			●							

Human

	Death	Acute	Intermediate	Chronic	Immunological/Lymphoreticular	Neurological	Reproductive	Developmental	Genotoxic	Cancer
	SYSTEMIC									
Inhalation			●	●		●	●	●	●	
Oral	●	●				●				
Dermal		●								

Animal

● Existing Studies

There are human data on inhalation exposure of carbon disulfide that provide information on acute and chronic systemic effects. There are also data on immunologic, neurologic, developmental, and reproductive effects. There are some limited data on the carcinogenic potential of carbon disulfide, but these are preliminary and confounded by multiple exposure problems. There are no oral exposure data from humans and only limited information on dermal exposures. The dermal data address the occupational hazard of blister formation following accidental exposure.

Animal data on inhalation exposure cover primarily intermediate systemic, neurological, developmental, and reproductive effects. One chronic study is available that examines systemic effects in rats. Oral data are limited to lethality data and information on acute systemic and neurological effects in mice and rats. Dermal data consist of some information regarding blister formation in humans and rabbits following dermal exposure.

2.10.2 Identification of Data Needs

Acute-Duration Exposure. The data on acute inhalation exposure in humans are limited. Transient respiratory difficulties were reported in some individuals following an accident in transporting carbon disulfide, but the actual exposures could not be determined (Spyker et al. 1982). Adverse effects including deaths were reported in a community in India following an accidental release of large amounts of carbon disulfide, hydrogen sulfide, and sulfuric acid from a viscose rayon plant (Kamat 1994). There are sufficient animal data to identify the nervous system (Liang et al. 1983; Magos and Jarvis 1970; Magos et al. 1974; Tarkowski et al. 1980), the cardiovascular system (Chandra et al. 1972; Tarkowski and Sobczak 1971), and the liver (Freundt et al. 1974a; Magos and Butler 1972; Magos et al. 1973) as target organs for acute inhalation exposure. Lethality (Gibson and Roberts 1972), decreased respiratory rate (Tarkowski and Sobczak 1971), and increased post-implantation loss (PAI 1991) have also been reported in acute studies. The acute studies have been done primarily in rodents; additional studies on other species, for example, primates, would be useful for generalizing to expected health effects in humans. No acute-duration inhalation MRL was derived since potential MRL values would not have been as protective of human health as the chronic-duration MRL. In addition, there is greater confidence in the chronic-duration MRL since it is based on human data.

Three case reports cited in Gosselin et al. (1984) indicate that half an ounce (concentration not specified) caused death following ingestion. There are no other human data on acute-duration oral exposure to carbon disulfide. There are sufficient animal data to identify the cardiovascular system (Hoffman and Klapperstück 1990) and the liver (Jones-Price et al. 1984b; Masuda et al. 1986) as targets for acute-duration oral exposure to carbon disulfide. Adverse effects including decreased body weight gain (Jones-Price et al. 1984b), hind-limb paralysis (Jones-Price et al. 1984b), decreased norepinephrine levels (Kanada et al. 1994), and developmental effects have also been reported. An acute-duration oral MRL of 0.01 mg/kg/day was derived based on dose-dependent decreases in the activities of liver microsomal drug-metabolizing enzymes in mice from the study by Masuda et al. 1986.

There are no human data on acute-duration dermal exposure to carbon disulfide. There is one acute study showing blister formation in rabbit ears when carbon disulfide was applied to the skin (Hueper 1936). Given that there is a possibility of dermal exposures in occupational settings as well as from contaminated water supplies near hazardous waste sites, additional data on dermal exposures would be useful. No pharmacokinetic data exist that show that dermal exposures to carbon disulfide result in patterns of distribution similar to those seen with inhalation or oral exposures. These kinds of data would be useful for projecting the similarities of target organs across routes as people near hazardous waste sites may be exposed by all three routes.

Intermediate-Duration Exposure. There are no human data on intermediate-duration inhalation exposures. Most occupational exposures are considered to be chronic-duration inhalation exposures, and there are no oral data for humans. The only dermal data for humans concern blisters on the fingers of rayon workers following 6 weeks of exposure (Hueper 1936).

There are animal data that identify the nervous system (Jirmanova and Lukas 1984; Juntunen et al. 1977; Liang et al. 1983; Merigan et al. 1985, 1988), the cardiovascular system (Antov et al. 1985), and the liver (Jarvisalo et al. 1977a; Tsuyoshi 1959; Wronska-Nofer 1972, 1973) as targets for intermediate-duration inhalation exposure to carbon disulfide. NOAELs and LOAELs exist for these exposures. Again, most of these data come from rodent studies, although some work has been done in monkeys (Merigan et al. 1988) and dogs (Lewey et al. 1941). The toxic effects are generally the same across species. There is some evidence that the reproductive (Tepe and Zenick 1984; Zenick et al. 1984) and developmental systems (Danielsson et al. 1984; Lehotzky et al. 1985; Tabacova and

Balabaeva 1980b; Tabacova et al. 1983; Yaroslavskii 1969) are also targets for intermediate-duration inhalation exposures. Lethality (Toxicogenics 1983c), decreased body weight (Rebert and Becker 1986), and hematologic (Toxicogenics 1983c) and musculoskeletal (Szendzikowski et al. 1974) effects have also been reported in intermediate-duration inhalation studies. No intermediate-duration MRL was derived since potential values would not have been as protective of human health as the chronic duration MRL. There are very few data on intermediate-duration oral exposures (Pilarska et al. 1973) and no data on intermediate-duration dermal exposures. Decreased body weight has been observed in rats following oral exposure to carbon disulfide for an intermediate-duration period (Hoffman and Klapperstück 1990). No intermediate-duration oral MRLs were derived because of a lack of reliable LOAELs and a lack of definitive target organs. Additional information on inhalation, oral, and dermal exposures would be useful for assessing the health risks to humans living near hazardous waste sites including identification of target organs.

Chronic-Duration Exposure and Cancer. There are data on chronic occupational exposures that identify the nervous system (Aaserud et al. 1988; Cassitto et al. 1978; Foa et al. 1976; Johnson et al. 1983; Peters et al. 1988; Putz-Anderson et al. 1983; Seppalainen and Tolonen 1974; Seppalainen et al. 1972; UK/IHSE 1981; Vasilescu 1976), the cardiovascular system (Cirla et al. 1972; Franco et al. 1982; Hernberg et al. 1970, 1971, 1973, 1976; Lieben et al. 1974; MacMahon and Monson 1988; Nurminen et al. 1982; Swaen et al. 1994; Tiller et al. 1968; Tolonen et al. l979), the liver (El-Sobkey et al. 1979; Mack et al. 1974; Rubin and Arieff 1945; Sidorowicz et al. 1980), and the eye (DeLaey et al. 1980; DeRouck et al. 1986; Raitta and Tolonen 1975; Raitta et al. 1974; Szymankova 1968) as primary targets for inhalation exposure to carbon disulfide. There are no data on chronic human oral or dermal exposures. Most of the occupational studies have limitations concerning the exposure measurements and concomitant exposures; some are limited by the methods used to assess the health effect end points. However, the Johnson et al. (1983) study, which evaluates nerve conduction velocity in workers exposed by inhalation to carbon disulfide, has been found acceptable for the derivation of a chronic-duration MRL. There is only one chronic inhalation study in rabbits (Cohen et al. 1959) and no chronic oral or dermal animal studies. Additional data concerning the effects of chronic low-level exposure to carbon disulfide following the inhalation, oral, and dermal routes would be useful to establish a dose-effect relationship for the major health effects, and to identify target organs for oral and dermal exposures. Long-term animal studies in several species, such as mice, rats, and monkeys, that investigate several dose points could be used to determine the neurotoxic mechanism of action.

There are no data suggesting an increased risk of cancer from exposure to carbon disulfide. Epidemiological data have been presented that indicate that there could be an association between carbon disulfide exposure and lymphocytic leukemia, but these studies had several problems with multiple comparisons, poor exposure variables, multichemical exposures, and no supporting animal or human data (Arp et al. 1983; Checkoway et al. 1984; Wilcosky et al. 1984). In addition, the lymphocytic leukemia may be associated with benzene impurity. A large population sample with better exposure data covering a minimum of 20 years would be helpful for addressing the repeatability of these findings. There are no studies regarding cancer in humans following oral or dermal exposure. In addition, there are no studies regarding cancer in animals via inhalation, oral, or dermal routes of exposure. A chronic animal bioassay would provide important information on effect and possibly on mechanism.

Genotoxicity. There are no human genotoxicity data for any route of exposure. Measurements of chromosomal aberrations or DNA adduct formation done on workers exposed via inhalation would be useful for assessing the genotoxic potential of carbon disulfide. Data obtained in this way, or from animals exposed *in vivo*, may provide evidence of some mechanism for the observed reproductive effects and, if correlated with exposures, could offer a potential biomarker of effect. This would provide some way of monitoring populations around hazardous waste sites. One *in vivo* animal study of the genotoxicity (chromosomal aberrations in the bone marrow of rats) of carbon disulfide reported negative results (NIOSH 1980). Bacterial mutagenicity assays (*Salmonella typhimurium* and *Escherichia coli)* have also generally been negative (Donner et al. 1981; Hadenstedt et al. 1979). Additional bacterial assays would not be useful at this time.

Reproductive Toxicity. There are human data that indicate that chronic inhalation exposure to carbon disulfide can affect the reproductive system in both males and females. In males, sperm morphology, hormone levels, and libido have been altered by occupational exposure to carbon disulfide (Lancranjan 1972; NIOSH 1983; Schrag and Dixon 1985; Vanhoorne et al. 1994; Wagar et al. 1981; Wyrobek 1983). In human females, menstrual irregularities have been associated with inhalation exposure to carbon disulfide, although more serious effects such as increased miscarriage and reduced fertility have not been universally noted (Cai and Bao 1981; Heinrichs 1983; Hemminki and Niemi 1982; Wang and Zhao 1987; Zhou et al. 1988; Zielhuis et al. 1984). There are no human data on the reproductive effects of oral or dermal exposure to carbon disulfide. Data on rats support the reproductive effects seen in humans after inhalation exposure only (Tepe and Zenick 1984; Zenick

et al. 1984). Additional reproductive studies on other species, such as mice, rabbits, dogs, and monkeys, would be useful to determine the dose-effect relationship between exposure and reproductive end points. It would also be useful to investigate reproductive organ pathology in a 90-day study of toxicity via inhalation, oral, and dermal exposures. There are no oral or dermal reproductive studies in animals.

Developmental Toxicity. There are no convincing human data that support an increased rate of congenital malformations in children born to mothers exposed by any route to carbon disulfide (Bao et al. 1991). Limitations of the Bao et al. (1991) study included the lack of exposure analyses and dose response assessments. There are data on rodents that suggest an increased fetotoxicity following inhalation exposure to carbon disulfide (Tabacova and Balabaeva 1980b; Yaroslavskii 1969). In addition, neurobehavioral effects have been reported in the offspring of exposed animal mothers (Lehotzky et al. 1985; Tabacova et al. 1983). Additional data from species other than rodents, for example, monkeys, would be useful for verifying that developmental effects are a result of exposure to this chemical. There are no animal data that provide information on the developmental effects of either oral or dermal exposure. There is evidence that carbon disulfide can cross the placenta and is distributed to the fetal brain, blood, liver, and eyes (Danielsson et al. 1984). Well-designed studies via inhalation, oral, and dermal exposures show that a dose-response relationship would be useful for determining the dose at which developmental effects could be expected to occur.

Immunotoxicity. There are no data that suggest that the immune system is a target for carbon disulfide exposure for any route or in any species. However, the results of one study indicated that the β-lipoprotein isolated from carbon disulfide exposed-workers (presumably exposed via inhalation) is antigenically identical to lipoproteins isolated from healthy nonexposed controls (Bobnis et al. 1976). There are no studies regarding immunological/lymphoreticular effects in humans following oral or dermal exposures. Also, there are no studies concerning immunological/lymphoreticular effects in animals following inhalation, oral, or dermal exposures. A 90-day study that investigates immune parameters should include routine immune function parameters (e.g., macrophage activity, T-cell activity, mitogen response, cell-mediated immune response) and immunopathology. This would be useful information for determining if there could be an immune system effect that has been overlooked.

Neurotoxicity. It is clear in both humans and animals that the nervous system is the primary target organ for carbon disulfide exposure for the inhalation route. There are behavioral (e.g., depression, decreased performance and memory), histopathological (e.g., polyneuropathy), and neurophysiological (e.g., decreased nerve conduction velocity) data in humans (Aaserud et al. 1988; Cassitto et al. 1978; Cirla et al. 1972; Foa et al. 1976; Johnson et al. 1983; Lancranjan 1972; Peters et al. 1986a, 1986b, 1988; Putz-Anderson et al. 1983; Seppalainen and Tolonen 1974; Seppalainen et al. 1972; Vasilescu 1976). The results of inhalation toxicity studies in animals have revealed neurophysiological (e.g., decreased nerve conduction velocity), neurochemical (e.g., altered noradrenalin and dopamine levels), and neurobehavioral (e.g., hind-limb paresis) (Frentik et al. 1994; Magos and Jarvis 1970; Tarkowski and Sobczak 1971; Wilmarth et al. 1993). A chronic-duration inhalation MRL was calculated on the basis of reduced motor nerve conduction velocity in humans (Johnson et al. 1983). There are no human data pertaining to neurological effects following oral or dermal exposures. There are limited animal data on neurotoxic effects (hind-limb paralysis) by the oral route (Jones-Price et al. 1984a, 1984b), and none for the dermal route (Dietzmann and Laass 1977). Studies investigating neurotoxic effects in animals following oral or dermal exposure would be useful for determining thresholds and dose-response relationships for neurotoxic effects.

Epidemiological and Human Dosimetry Studies. There are many epidemiological studies that address the effects of inhalation exposure to carbon disulfide. These studies include both occupational- and community-based cohorts and have investigated neurological (Aaserud et al. 1988; Cassitto et al. 1978; Cirla et al. 1972; Foa et al. 1976; Johnson et al. 1983; Peters et al. 1988; Putz-Anderson et al. 1983; Seppalainen and Tolonen 1974; Seppalainen et al. 1972), cardiovascular (El-Sobkey et al. 1979; Franco et al. 1982; Hernberg et al. 1970, 1971, 1973, 1976; Lieben et al. 1974; MacMahon and Monson 1988; Nurminen and Hernberg 1985; Nurminen et al. 1982; Rubin and Arieff 1945; Sidorowicz et al. 1980; Sugimoto et al. 1978; Tiller et al. 1968; Tolonen et al. 1975, 1979; UK/HSE 1981), and reproductive (Cai and Bao 1981; Heinrichs 1983; Hemminki and Niemi 1982; Lancranjan 1972; NIOSH 1983; Wagar et al. 1981; Wang and Zhao 1987; Zhou et al. 1988) effects. These studies have found positive associations between carbon disulfide exposure and adverse health effects. These findings are not universal, however. The epidemiological studies have the limitation of poor exposure measurements that are not individualized to the study participants. In addition, the exposures postulated to have occurred in these cohorts cover a wide range of levels, making extrapolations from one study to another difficult; many of these studies also documented concomitant exposures to other chemicals, most notably hydrogen sulfide. A principle components

exposure index has been developed for simultaneous exposures to hydrogen sulfide and carbon disulfide by Vanhoorne et al. (1995). Nonetheless, they provide data on human health effects, and the weight of the evidence is that the environment where carbon disulfide is used exerts a range of effects at different exposure levels. Clearly, occupational workers, as well as communities around hazardous waste sites or point-emission sources, are at risk for exposure to levels of carbon disulfide that have been associated with adverse health effects. The biggest drawback in the existing studies is the lack of the ability to establish a dose relationship between exposure and effect. More precise measurements of exposure, control of exposure to other chemicals, and long-term follow-up of occupational cohorts may lead to a better understanding of the dose-effect of carbon disulfide. Monitoring of populations around hazardous waste sites where carbon disulfide is known to be present would be useful, providing specific biomarkers of exposure could be identified.

Biomarkers of Exposure and Effect

Exposure. Biomarkers of exposure for carbon disulfide include levels of carbon disulfide in exhaled breath (Philips 1992), blood (WHO 1979), and urine (Djuric 1967; McKee et al. 1943) and carbon disulfide thiometabolites in the urine (Beauchamp et al. 1983; Campbell et al. 1985). These measurements can indicate whether acute-, intermediate-, or chronic-duration exposure to carbon disulfide has occurred. The presence of carbon disulfide in various biological media is the most specific biomarker of exposure. However, few studies have been able to demonstrate a straightforward correlation between the above parameters and actual carbon disulfide exposure. There are two possible explanations for this observation. The first one is that too many variables tend to confound the measurements of carbon disulfide in the blood, breath, and urine of exposed individuals, rendering these biomarkers somewhat unreliable for quantitative evaluation. The second possibility is that the limitations of the protocols and analytical methods employed for measuring carbon disulfide in biological media have largely precluded acquiring data related to ascertaining the reliability of these biomarkers. Therefore, with the development of new and more sensitive methods, blood, breath, and urine levels of carbon disulfide may become even more useful biomarkers of exposure. More quantitative, correlative studies are necessary in order to assess this possibility.

At the present time, the biomarkers that correlate best with exposure are metabolite levels in the urine (Baselt 1980; Beauchamp et al. 1983; Campbell et al. 1985; Lieben 1974i; WHO 1986). The iodine-azide and the TTCA tests, which measure the presence of urinary carbon disulfide metabolites, have

been shown to correlate well with actual exposure. However, the iodine-azide test is nonspecific (Dox et al. 1992). TTCA is produced in humans after exposure to Antabuse and in rats after exposure to Captan (Cox et al. 1992). Moreover, other investigations are necessary in order to determine whether the interaction of carbon disulfide with other substances (such as hydrogen sulfide, drugs, carbon tetrachloride, malathion, and alcohol), disease states, and variations in diet and in individual metabolism, as well as other factors, could confound the results of the iodine-azide test and the TTCA test for carbon disulfide exposure. Baseline urine, breath, and blood samples are necessary to correct for non-workplace exposures For exposures around hazardous waste sites, the influence of workplace exposures must also be corrected for in this manner

Effect. Few specific biomarkers of effect have been identified for carbon disulfide. However, many biological parameters have been tentatively linked with carbon disulfide's effects on certain enzyme systems, on the liver and the kidneys, and on the nervous, cardiovascular, reproductive, and endocrine systems (Baselt 1980; Beauchamp et al. 1983; Cai and Bao 1981; Campbell et al. 1985; Djuric 1967; El-Sobkey et al. 1979; Heinrichs 1983; Hernberg et al. 1971; Lancranjan 1972; Lewey et al. 1941; Lieben 1974; McKee et al. 1943; Prerovska and Drdkova 1967; Toyama and Sakurai 1967; Valentine et al. 1993; Wang and Zhao 1987; WHO 1979, 1981, 1986; Zhou et al. 1988). No studies attempting to quantitatively correlate these parameters with carbon disulfide effects are available. CT-scan (computerized tomography) and electromyographical parameters as well as measurements of nerve conduction velocity may serve as indicators of nervous system effects (Beauchamp et al. 1983; WHO 1981). High serum lipid content (in particular cholesterol) may be linked with cardiovascular effects (El-Sobkey et al. 1979; Lewey et al. 1941; Prerovska and Drdkova 1967; Toyama and Sakurai 1967). Reduction in steroid hormone synthesis may indicate early reproductive system effects (Cai and Bao 1981; Heinrichs 1983; Lancranjan 1972; Wang and Zhou 1987; Zhou et al. 1988). Also higher plasma creatinine levels and elevated blood sugar could signal renal dysfunction due to carbon disulfide exposure (Hernberg et al 1971; Lewey et al. 1941). Changes in various enzyme systems (for example, lowered succinic-oxidase activity) may prove useful as biomarkers of effect (McKee et al. 1943). The effect of carbon disulfide on membrane spectrin of nerve and blood membranes should be investigated further (Valentine et al. 1993). However, changes in these biological parameters are not specific to carbon disulfide exposure, and future studies are needed to determine the extent to which they quantitate the results of carbon disulfide exposure. In addition, these studies should evaluate whether such biological parameters may serve as reliable and accurate biomarkers of carbon disulfide exposure. However, since many different things can cause these effects, biomarkers may never be

specific for exposure to carbon disulfide. Nervous system effects are well documented for inhalation exposures but not for oral or dermal exposures.

Absorption, Distribution, Metabolism, and Excretion. There are human and animal data that address the absorption, distribution, metabolism, and excretion of carbon disulfide following inhalation exposure (Abramova 1966; Bond and DeMatteis 1969; Brieger 1967; Cohen et al. 1958, 1959; Dalvi and Neal 1978; Dalvi et al. 1974, 1975; DeMatteis 1974; DeMatteis and Seawright 1973; Djerassi and Lumbroso 1968; Djuric 1967; Freundt et al. 1975; McKee et al. 1943; McKenna and Distefano 1977a, 1977b; Okayama et al. 1988; Pergal et al. 1972a, 1972b; Santodonato et al. 1985; Snyderwine and Hunter 1987; Soucek 1957; Strittmatter et al. 1950; Teisinger 1974; Van Doorn et al. 1981a, 1981b; Vasak and Kopecky 1967). Data indicate rapid and extensive absorption of inhaled carbon disulfide, distribution through the body, and primary excretion by exhalation. Carbon disulfide is metabolized by cytochrome P-450 to an unstable oxygen intermediate that in turn can either degrade to sulfur and carbonyl sulfide or hydrolize to sulfur and monothiocarbamate. Biotransformation of carbon disulfide in humans exposed by the inhalation route causes TTCA, OTCA, and thiourea to be excreted in the urine, and carbonyl sulfide and carbon dioxide in the breath. The data that exist for humans are largely supported by animal studies (rabbits and dogs) for this route. However, there are very few animal and human data regarding the pharmacokinetics of carbon disulfide following oral or dermal exposure, making an assessment of relative rates very difficult (Cohen et al. 1958; DeMatteis and Seawright 1973; Dutkiewicz and Baranowska 1967). The limited data indicate that a range fraction of orally administered carbon disulfide is absorbed by rats. Carbon disulfide is appreciably absorbed via the dermal route in rabbits. Animal data suggest that there are two major pathways. Steady-state phenomena do play a role in the retention and excretion of carbon disulfide, with less exposed individuals retaining more of the chemical than chronically exposed individuals (Beauchamp et al. 1983). Additional information regarding the pharmacokinetics of carbon disulfide following oral and dermal exposure would be useful. The use of TTCA as a relatively selective biological marker of exposure to carbon disulfide is now well accepted (ACGIH 1994). Since the critical TTCA concentration is tied to the TLV-TWA for inhalation, more research is required to ensure that the TLV-TWA is truly protective.

Comparative Toxicokinetics. Both human and animal data indicate that the target organs for carbon disulfide are similar across species (Cohen et al. 1958; DeMatteis and Seawright 1973; Dutkiewicz and Baranowska 1967; Freundt et al. 1975; McKee et al. 1943; Soucek 1957; Teisinger

and Soucek 1949; Toyama and Kusano 1953). There are no studies that directly compare the toxicokinetics across species. Most of the animal studies on toxicity end points have used high doses. The studies in rats, mice, and rabbits have generally been consistent in their conclusions regarding the pharmacokinetics of carbon disulfide. Data from species other than rodents would also be useful for determining the species most comparable to humans, so that animal toxicity data can be better evaluated. No striking differences between the results of rodent studies and those from human studies were noted except that sulfate excretion is far more important in animals than in humans except in the latter for exposure to high doses of carbon disulfide (Strittmatter et al. 1950). Additional information on the comparative pharmacokinetics following exposure from the oral and dermal routes would be useful, as most of the data currently available are from inhalation studies. The volatility of carbon disulfide may well affect kinetic parameters measured in dermal exposures, and metabolic parameters following oral exposures could differ from those following inhalation exposure.

Methods for Reducing Toxic Effects. Reduction of absorption of carbon disulfide may result from treatment with activated carbon and with sodium bicarbonate solutions (Dreisbach and Robertson 1987; Stutz and Janusz 1988). Some of the treatment methods currently available for use in carbon disulfide exposure, such as activated charcoal, gastric lavage, and induced emesis, support the survival of the exposed individual. Other treatments, such as reversal of central nervous system depression by administration of caffeine and sodium benzoate or providing excess zinc or copper salts, may provide only temporary relief of symptoms (Morgan 1982). Additional information on the ultimate mechanism of carbon disulfide toxicity is needed before insights may be gained regarding treatment of exposure victims.

2.10.3 On-going Studies

No studies of carbon disulfide were reported in the Federal Research in Progress database (FEDRIP 1995).

A 13-week inhalation study in rats sponsored by the National Toxicology Program (NTP) has been completed, but the results were not published as of March 1996. The NTP has also reported that an inhalation carcinogenicity study in rats has been planned, but no details on this study are available. Preliminary reports on NTP-funded genotoxicity studies indicate positive results in a sister chromatid

exchange assay (*in vitro*) and negative results in both a chromosome aberration assay (*in vitro*) and in two *Salmonella* tests. No further data on these studies were available.

The following studies, reported in Federal Research in Progress (FEDRIP 1989), have not been published as of this date.

K. Boekelheide (Brown University) is studying the mechanism involved in testicular injury in rats from exposure to carbon disulfide and other industrial and environmental toxicants.

B. Gold (Rutgers University Medical and Dental School) is conducting studies on the neurotoxicity of carbon disulfide in rats.

R. Rubin (Johns Hopkins University) is evaluating the interaction of alcohols and solvents on the toxicity and kinetics of carbon disulfide in rats, mice, and hamsters.

D.W. Lynch (NOSH) is studying the adverse effects of carbon disulfide on the cardiovascular system in rats.

3. CHEMICAL AND PHYSICAL INFORMATION

3.1 CHEMICAL IDENTITY

The chemical identity of carbon disulfide is located in Table 3-l.

3.2 PHYSICAL AND CHEMICAL PROPERTIES

The physical and chemical properties of carbon disulfide are located in Table 3-2.

TABLE 3-1. Chemical Identity of Carbon Disulfide

Characteristic	Information	Reference
Chemical name	Carbon disulfide	HSDB 1995
Synonym(s)	Carbon bisulphide; carbon disulphide; carbon sulfide; carbon sulphide; dithiocarbonic anhydride; sulphocarbonic anhydride	HSDB 1995
Registered trade name(s)	Weeviltox®	HSDB 1995
	Caswell No. 162®	HSDB 1995
Chemical formula	CS_2	
Chemical structure	S=C=S	
Identification numbers:		
CAS registry	75-15-0	HSDB 1995
NIOSH RTECS	FF6650000	HSDB 1995
EPA hazardous waste	P022 (pure)	HSDB 1995
	F-005 (as a mixture component)	
OHM/TADS	7216633	HSDB 1995
DOT/UN/NA/IMCO shipping	UN 1131; IMCO 6.1	HSDB 1995
HSDB	52	HSDB 1995
NCI	C04591	HSDB 1995

CAS = Chemical Abstracts Services; DOT/UN/NA/IMCO = Department of Transportation/United Nations/North America/International Maritime Dangerous Goods Code; EPA = Environmental Protection Agency; HSDB = Hazardous Substances Data Bank; NCI = National Cancer Institute; NIOSH = National Institute for Occupational Safety and Health; OHM/TADS = Oil and Hazardous Materials/ Technical Assistance Data System; RTECS = Registry of Toxic Effects of Chemical Substances

TABLE 3-2. Physical and Chemical Properties of Carbon Disulfide

Property	Information	Reference
Molecular weight	76.14	Windholz 1983
Color	Clear, colorless, or faintly yellow	Sax and Lewis 1987
	Colorless	Windholz 1983
Physical state	Highly refractive, mobile liquid	Windholz 1983
Melting point	-110.8°C	Weast 1989
	-111.5°C	HSDB 1995
Boiling point	46.5°C (at 760 torr)	Windholz 1983
Density		
at 15°C	1.27055 g/mL	Windholz 1983
at 20°C	1.2632 g/mL	Windholz 1983
at 30°C	1.2481 g/mL	Windholz 1983
Odor	Purest distillates have sweet, pleasing, and ethereal odor; commercial and reagent grades are foul smelling	Flick 1985; Windholz 1983
Odor threshold:		
Water	0.0026 mg/L (faint odor)	Verschueren 1983
Air	0.31–0.65 mg/m^3 (0.1–0.2 ppm)	ACGIH 1986
	low = 0.0243 mg/m^3 (0.008 ppm)	Ruth 1986
	high = 23.1 mg/m^3 (7.39 ppm)	Ruth 1986
	0.31 mg/m^3 (0.1 ppm) (response in 50% of subjects)	MCA 1968
	0.65 mg/m^3 (0.2 ppm) (response in 100% of subjects)	MCA 1968
	0.05 mg/m^3 (0.02 ppm) (perception in humans)	Verschueren 1983
	0.04 mg/m^3 (0.01 ppm) (nonperception with adverse reflex response in humans)	Verschueren 1983
Solubility:		
Water		
at 20°C	2940 mg/L	Windholz 1983
at 22°C	2300 mg/L	Verschueren 1983
Organic solvents	Miscible with anhydrous methanol, ethanol, ether, benzene, chloroform, carbon tetrachloride, and oils	Windholz 1983

TABLE 3-2. Physical and Chemical Properties of Carbon Disulfide (*continued*)

Property	Information	Reference
Partition coefficients:		
Log K_{ow}	1.84–2.16 (calculated)	Verschueren 1978
Log K_{oc}	1.80	HSDB 1995
Vapor pressure		
at 10°C	127.0 mmHg	Flick 1985
at 10°C	200 mmHg	Verschueren 1983
at 20°C	260 mmHg	Verschueren 1983
at 20°C	297.5 mmHg	Timmerman 1978
at 25°C	352.6 mmHg	Worthing 1987
at 30°C	430 mmHg	Verschueren 1983
Henry's law constant	1.22×10^{-2} atm m^3/mol	EPA 1981
Autoignition temperature	100°C	Windholz 1983; Sax and Lewis 1987
	125–135°C	Worthing 1987
Flashpoint	-30°C (closed cup)	NFPA 1986; Sax and Lewis 1987; Windholz 1983
Flammability limits in air	1–50% (v/v)[a] (explosive range)	Flick 1985; Windholz 1983
	1.3–50%	NFPA 1986; Weiss 1980
Conversion factors	0.32 ppm = 1 mg/m^3	Beauchamp et al. 1983
Explosive limits	lower = 1% upper = 50%	OHMTADS 1995

[a]v/v = percent "volume in volume," which expresses the number of milliliters of pure analyte vapor in 100 milliliters of air mixture (ACGIH 1995)

4. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

4.1 PRODUCTION

Carbon disulfide was first manufactured commercially around 1880 (Timmerman 1978). Until about 1950, the primary industrial-scale production method was by heating charcoal to 750-900°C in the presence of vaporized low-ash sulfur. However, in the United States, extensive research was devoted to finding alternative sources of carbon disulfide, and by 1965 the coal-burning method was largely replaced by a reaction involving natural gas hydrocarbons such as methane, ethane, and ethylene (Timmerman 1978; Windholz 1983). Carbon disulfide is normally available both in technical and reagent grades (up to 99.9% pure with a trace benzene contaminant) (Sax and Lewis 1987; Timmerman 1978) and in alkali (thiocarbonates) as emulsions or solutions for soil treatment (Spencer 1982; Worthing 1987). Trade names for preparations include Weeviltox and Caswell No. 162 (HSDB 1995).

Trends in carbon disulfide production have closely paralleled those of the viscose rayon industry, one of its largest users (HSDB 1995; Mannsville Chemical Products Corp. 1985; Timmerman 1978; WHO 1981). Production increased by nearly 50% between 1941 and 1969, from 242,000 to 362,000 metric tons. This increase was partly due to a sudden rise in demand for carbon tetrachloride, an intermediate in the production of fluorocarbon propellants and refrigerants; carbon disulfide is used in the production of carbon tetrachloride. The 1969 production level remained relatively stable until about 1974 when it declined sharply to the 1975 level of 217,000 metric tons (Timmerman 1978). Carbon disulfide production levels continued to decline, with fluctuations, to 168,000 metric tons in 1984 (Mannsville Chemical Products Corp. 1985; Timmerman 1978). In 1985, production was estimated to be 143,000 metric tons (Mannsville Chemical Products Corp. 1985). No information was found on production levels after 1985.

Because of a long-term decline in the demand for viscose rayon and cellophane and restrictions on the use of fluorocarbon propellants, future production levels of carbon disulfide are uncertain. However, it is expected that demand for this chemical in many other specialty areas will continue at relatively stable levels (Mannsville Chemical Products Corp. 1985; Timmerman 1978).

In the United States, the principal manufacturers of carbon disulfide include Lenzing Fibers Corp., ELF Atochem N.A. Inc., and PPG Industries (TRI93 1995). For additional information on companies that manufacture or process carbon disulfide, refer to Table 4-l. According to the 1993 Toxics Release Inventory (TRI), 80 facilities manufactured or processed carbon disulfide in 1993, 9 fewer than in 1991 (TRI93 1995). Seventy-nine (79) of these facilities reported the maximum amount of carbon disulfide that they would have on site. These data are listed in Table 4-l. The TRI data should be used with caution since only certain types of facilities are required to report. Carbon disulfide is also produced in 14 foreign countries, including 4 producers in Canada, 7 in South and Central America, 9 in Asia, 8 in Europe, and 1 in Australia (CIS 1989). The total annual capacity of the 13 principal foreign manufacturers in 1977 was 911,000 metric tons.

4.2 IMPORT/EXPORT

Imports of carbon disulfide have fallen at a fairly steady rate from 2,700 metric tons in 1980 to 1,400 metric tons in 1985. Exports, on the other hand, fell sharply from 5,900 metric tons in 1980 to 900 metric tons in 1982. Exports continued to decline to 450 metric tons in 1983 and then rose to 1,400 metric tons in 1984 (Mannsville Chemical Products Corp. 1985). No information was found on export levels after 1985.

4.3 USE

Carbon disulfide has been an important industrial chemical since the 1800s because of its many useful properties, including its ability to solubilize fats, rubbers, phosphorus, sulfur, and other elements (Sine 1989; Timmerman 1978; Windholz 1983). Because of its ability to dissolve phosphorus, it was once widely used to produce matches but was later replaced by another chemical. Carbon disulfide’s fat solvent properties also made it indispensable in preparing fats, lacquers, and camphor; in refining petroleum jelly and paraffin; and in extracting oil from bones, palmstones, olives, and rags. It was also used in processing India rubber sap from tropical trees. In all of these extraction processes, however, carbon disulfide has been replaced by other solvents (Davidson and Feinleib 1972).

Its fat, rubber, and metal solvent properties have made carbon disulfide highly suitable for a variety of other continuing industrial applications including the following: vulcanization and manufacture of

TABLE 4-1. Facilities That Manufacture or Process Carbon Disulfide

Facility	Location[a]	Range of maximum amounts on site in pounds	Activities and uses
GULF STATES STEEL INC.	GADSDEN, AL	1,000-9,999	Produce; As an impurity
GE CO.	BURKVILLE, AL	0-99	Produce; As an impurity
AKZO NOBEL CHEMICALS INC.	AXIS, AL	No data	Produce; For on-site use/processing; For sale/distribution; As a reactant; As a chemical processing aid
COURTAULDS FIBERS INC.	AXIS, AL	100,000-999,999	As a reactant
WITCO CORP.	PHENIX CITY, AL	100-999	Produce; As a by-product
VISKASE CORP.	OSCEOLA, AR	100,000-999,999	As a chemical processing aid
COLUMBIAN CHEMICALS CO.	EL DORADO, AR	0-99	Produce; As a by product
HICKSON KERLEY INC.	SAHUARITA, AZ	100,000-999,999	As a reactant
MINEREC INC.	TUCSON, AZ	100,000-999,999	As a reactant
ZENECA INC.	RICHMOND, CA	100,000-999,999	As a reactant
AMVAC CHEMICAL CORP.	LOS ANGELES, CA	100,000-999,999	As a reactant
VANDERBILT CHEMICAL CORP.	BETHEL, CT	100,000-999,999	As a reactant
UNIROYAL CHEMICAL CO. INC.	CT	100,000-999,999	As a reactant
STAR ENT.	DELAWARE CITY, DE	10,000-99,999	As a chemical processing aid
VININGS IND. INC.	MARIETTA, GA	100,000-999,999	As a reactant
HERCULES INC.	BRUNSWICK, GA	10,000-99,999	As a reactant
TEXTILE RUBBER & CHEMICAL CO.	GA	100,000-999,999	As a reactant
VISKASE CORP.	BEDFORD PARK, IL	100,000-999,999	As a chemical processing aid
AMOCO	WOOD RIVER, IL	100,000-999,999	As a reactant
BF GOODRICH	HENRY, IL	100,000-999,999	As a reactant
TEEPAK INC.	DANVILLE, IL	100,000-999,999	As a reactant; As a chemical processing aid
UNO-VEN CO.	IL	0-99	Produce; As a by-product
BETHLEHEM STEEL CORP.	BURNS HARBOR, IN	1,000-9,999	Produce; As a by-product
GE CO.	MOUNT VERNON, IN	0-99	Produce; As a by-product
FLEXEL INDIANA INC.	COVINGTON, IN	100,000-999,999	As a chemical processing aid
COLUMBIAN CHEMICALS CO.	ULYSSES, KS	0-99	Produce; As a by-product
FLEXEL INC.	TECUMSEH, KS	100,000-999,999	As a reactant
VANDERBILT CHEMICAL CORP.	MURRAY, KY	100,000-999,999	As a reactant
UNIROYAL CHEMICAL CO. INC.	GEISMAR, IA	100,000-999,999	As a reactant
CITGO PETROLEUM CORP.	LAKE CHARLES, LA	1,000-9,999	Produce; As a by-product
RHONE-POULENC BASIC CHEMICALS	BATON ROUGE, LA	100,000-999,999	Ancillary uses
CABOT CORP.	VILLE PLATTE, LA	100-999	Produce; As a by-product
DEGUSSA CORP.	LOUISA, LA	0-99	Produce; As a by-product

TABLE 4-1. Facilities That Manufacture or Process Carbon Disulfide (continued)

Facility	Location[a]	Range of maximum amounts on site in pounds	Activities and uses
WITCO CORP.	TAFT, LA	100,000-999,999	As a reactant
MARINE SHALE PROCESSORS, INC.	AMELIA, LA	1,000-9,999	As a reactant
CABOT CORP.	FRANKLIN, LA	100-999	Produce; As a by-product
COLUMBIAN CHEMICALS	CENTERVILLE, LA	0-99	Produce; As a by-product
KOCH IND. INC.	MN	1,000-9,999	Produce; As a by-product
BAYER CORP.	KANSAS CITY, MO	100,000-999,999	As a reactant
PETROLITE CORP	SAINT LOUIS, MO	10,000-99,999	As a reactant
BUCKMAN LABS. INC.	CADET, MO	100,000-999,000	As a reactant
AMERADA HESS CORP.	PURVIS, MS	1,000-9,999	As a chemical processing aid
MONTANA SULPHUR & CHEMICAL CO.	BILLINGS, MT	0-99	Produce; As a by-product; As a reactant
DU PONT	DEEPWATER, NJ	100,000-999,999	As a reactant
MERCK & CO. INC.	RAHWAY, NJ	100,000-999,999	As a reactant
3M	TONAWANDA, NY	100,000-999,999	As a reactant
GOODYEAR TIRE & RUBBER CO.	NIAGARA FALLS, NY	100,000-999,999	As a reactant
FERRO CORP.	WALTON HILLS, OH	10,000-99,999	As a reactant
ZENECA INC.	PERRY, OH	100,000-999,999	As a reactant
LUBRIZOL CORP.	PAINESVILLE, OH	10,000-99,999	As a reactant
NYLONGE CORP.	ELYRIA, OH	100,000-999,999	As a reactant
WITCO CORP.	PONCA CITY, OK	100-999	Produce; As a by-product
FLEXSYS AMERICA LP	MONOGAHELA, PA	100,000-999,999	As a chemical processing aid
SOUTHERN WATER TREATMENT CO.	GRENVILLE, SC	100,000-999,999	As a reactant
HODGSON CHEMICALS INC.	ROCK HILL, SC	100,000-999,999	As a reactant
NORTH AMERICAN RAYON CORP.	ELIZABETHTON, TN	100,000-999,999	As a reactant; As a chemical processing aid
LENZING FIBERS CORP.	LOWLAND, TN	1.000,000-9,999,999	As a reactant
ALCO CHEMICAL	CHATTANOOGA, TN	100,000-999,999	As a reactant
VISKASE CORP.	LOUDON, TN	100,000-999,999	As a chemical processing aid
SPONTEX INC.	COLUMBIA, TN	100,000-999,999	As a reactant
DEGUSSA CORP.	ARANSAS PASS, TX	0-99	Produce; As a by-product
DOW CHEMICAL CO.	FREEPORT, TX	0-99	Produce; As an impurity
CABOT CORP.	PAMPA, TX	0-99	Produce; As a by-product
J. M. HUBER CORP.	BAYTOWN, TX	0-99	Produce; As a by-product
GOODYEAR TIRE & RUBBER CO.	HOUSTON, TX	0-99	Produce; As a by-product
RHONE-POULENC BASIC CHEMICALS	HOUSTON, TX	10,000-99,999	Ancillary uses

TABLE 4-1. Facilities That Manufacture or Process Carbon Disulfide (continued)

Facility	Location[a]	Range of maximum amounts on site in pounds	Activities and uses
LUBRIZOL CORP.	DEER PARK, TX	10,000-99,999	As a reactant
J. M. HUBER CORP.	BORGER, TX	0-99	Produce; As a by-product
ELF ATOCHEM N.A. INC.	BEAUMONT, TX	1,000,000-9,999,999	Produce; As a by-product
BAKER PERFORMANCE CHEMICALS	DAYTON, TX	10,000-99,999	As a formulation component
WITCO CORP.	SUNRAY, TX	0-99	Produce; As a by-product
J. M. HUBER CORP.	ORANGE, TX	0-99	Produce; As a by-product
DU PONT	RICHMOND, VA	100,000-999,999	As a reactant; As a chemical processing aid
HICKSON KERLEY INC.	KENNEWICK, WA	100,000-999,999	As a reactant
3M	WI	100,000-999,999	As a reactant
PPG IND. INC.	NEW MARTINSVILLE, WV	1,000,000-9,999,999	Produce; For sale/distribution
COLUMBIAN CHEMICALS CO.	PROCTOR, WV	0-99	Produce; As a by-product
CABOT CORP.	WAVERLY, WV	0-99	Produce; As a by-product
AC & S INC.	NITRO, WV	10,000-99,999	As a reactant
MONSANTO CO.	NITRO, WV	100,000-999,999	As a reactant

Source: TRI93 1995

[a]Post office state abbreviations used

NA = not available

rubber and rubber accessories; production of resins, xanthates, thiocyanates, plywood adhesives, and flotation agents; solvent and spinning-solution applications primarily in the manufacture of rayon; polymerization inhibition of vinyl chloride; conversion and processing of hydrocarbons; petroleum-well cleaning; brightening of precious metals in electroplating; thin film deposition of nickel; as an agent to increase corrosion and wear-resistance in metals; rust removal from metals; and removal and recovery of metals and other elements from waste water and other media (Davidson and Feinleib 1972; EPA 1978a; Sine 1989; WHO 1981; Windholz 1983; Worthing 1987). It has also been used in industry as a means to promote sulfation in the synthesis of rare earth sulfides used in semiconductors, as a regenerator for transition metal sulfide catalysts, as a development restrainer in photography and lithography, and as a solvent to remove printing on recycled plastics (Timmerman 1978).

Carbon disulfide's most important industrial use, however, has been in the manufacture of regenerated cellulose rayon by the viscose process (viscose rayon) and of cellophane (Davidson and Feinleib 1972; EPA 1978a; NIOSH 1977; Timmerman 1978; WHO 1981). In 1974, over 80% of the carbon disulfide manufactured was used to make viscose rayon and cellophane (Austin 1974). This proportion fell to 50% in 1984, but the rayon and cellophane uses still accounted for the greatest fraction of carbon disulfide production (Mannsville Chemical Products Corp. 1985). Since 1989, the consumption of carbon disulfide in the production of carbon tetrachloride has increased to 38%, while the rayon industry's consumption has dropped to 34% (HSDB 1995).

Another principal industrial use for carbon disulfide has been as a feedstock for carbon tetrachloride production (Mannsville Chemical Products Corp. 1985; NIOSH 1977; Timmerman 1978). While only 10% of U.S. carbon disulfide production was used to produce carbon tetrachloride in 1960, this increased to 32% in 1974, largely because of a rapid increase in the demand for carbon tetrachloride for the production of fluorocarbon propellants and refrigerants (Timmerman 1978). Although most chemical manufacturers have switched to methanol as a raw material for carbon tetrachloride, beginning in 1985, Akzo America, Inc., continued to use carbon disulfide for this purpose (Mannsville Chemical Products Corp. 1985). Beginning in 1989, 38% of the carbon disulfide produced was used to manufacture carbon tetrachloride (HSDB 1995).

In the food industry, carbon disulfide has been used to protect fresh fruit from insects and fungus during shipping, in adhesives for food packaging, and in the solvent extraction of growth inhibitors (Timmerman 1978).

In agriculture, carbon disulfide has been widely used as a fumigant to control insects in stored grain, normally when mixed with carbon tetrachloride to reduce the fire hazard (Sine 1989; Worthing 1987). It has also be used to remove botfly larva infestations from the stomachs of horses and ectoparasites from swine (Rossoff 1974). Use of carbon disulfide as a grain fumigant was voluntarily cancelled after 1985 (EPA 1985a). An intensive specialty use is to desorb charcoal sampling tubes in NIOSH methods for airborne organics (NIOSH 1984b). Carbon disulfide is used extensively in research laboratory chemical synthetics methods (Dunn and Rudolf 1989).

In 1989, the estimated distribution of carbon disulfide utilization was as follows: 34% of production went to manufacture viscose rayon, 6% to produce cellophane, 38% to produce carbon tetrachloride, 7% to produce rubber chemicals, and 15% to produce pesticides and to solubilize waxes and oils (HSDB 1995). Future use patterns remain uncertain, although it is expected that less may be used to produce viscose rayon, cellulose, and carbon tetrachloride, products for which the demand has declined and for which alternate production processes may be found (HSDB 1995; Mannsville Chemical Products Corp. 1985; Timmerman 1978). Unless substitutes for carbon disulfide are found, its use levels may depend largely on relative import and export levels of textiles and apparel (Mannsville Chemical Products Corp. 1985). Carbon disulfide use for many other specialty industrial purposes is expected to continue (HSDB 1995; Timmerman 1978).

4.4 DISPOSAL

Carbon disulfide is a very flammable liquid that bums to produce carbon dioxide and sulfur dioxide. Therefore, it is a good candidate for controlled incineration, provided that a sulfur dioxide scrubber is used. Some methods proposed by the EPA (HSDB 1995) include liquid injection incineration at a temperature ranging from 650°C to 1,600ºC, rotary kiln incineration at a temperature range of 820-1,600ºC, and fluidized bed incineration at a temperature range of 450-980°C. Carbon disulfide can be removed from waste water by air stripping (HSDB 1995). Adsorption to activated coal with hydrogen sulfide in the absence of free oxygen yields a process that can regenerate large percentages of sulfur for reuse (HSDB 1995). It is not recommended that landfills be used as a disposal method because of the high flammability of this compound (HSDB 1995). No information was found on quantities and locations of disposal. The EPA CERCLA guideline for reportable quantities is 100 pounds (EPA 1995m).

5. POTENTIAL FOR HUMAN EXPOSURE

5.1 OVERVIEW

The primary disposition of carbon disulfide in the environment is related to its use as an industrial solvent and chemical intermediate. Releases from industrial processes are almost exclusively to the atmosphere. Releases of the compound to surface waters and soils are expected to partition rapidly to the atmosphere through volatilization. Hydrolysis and biodegradation do not appear to be important processes in determining the environmental fate of carbon disulfide. It has been detected at generally low levels in ambient air, surface water, groundwater, drinking water, food products, and human milk. Concentrations in environmental media are greatest near source areas (e.g., industrial point sources, oceans and marshes, volcanoes).

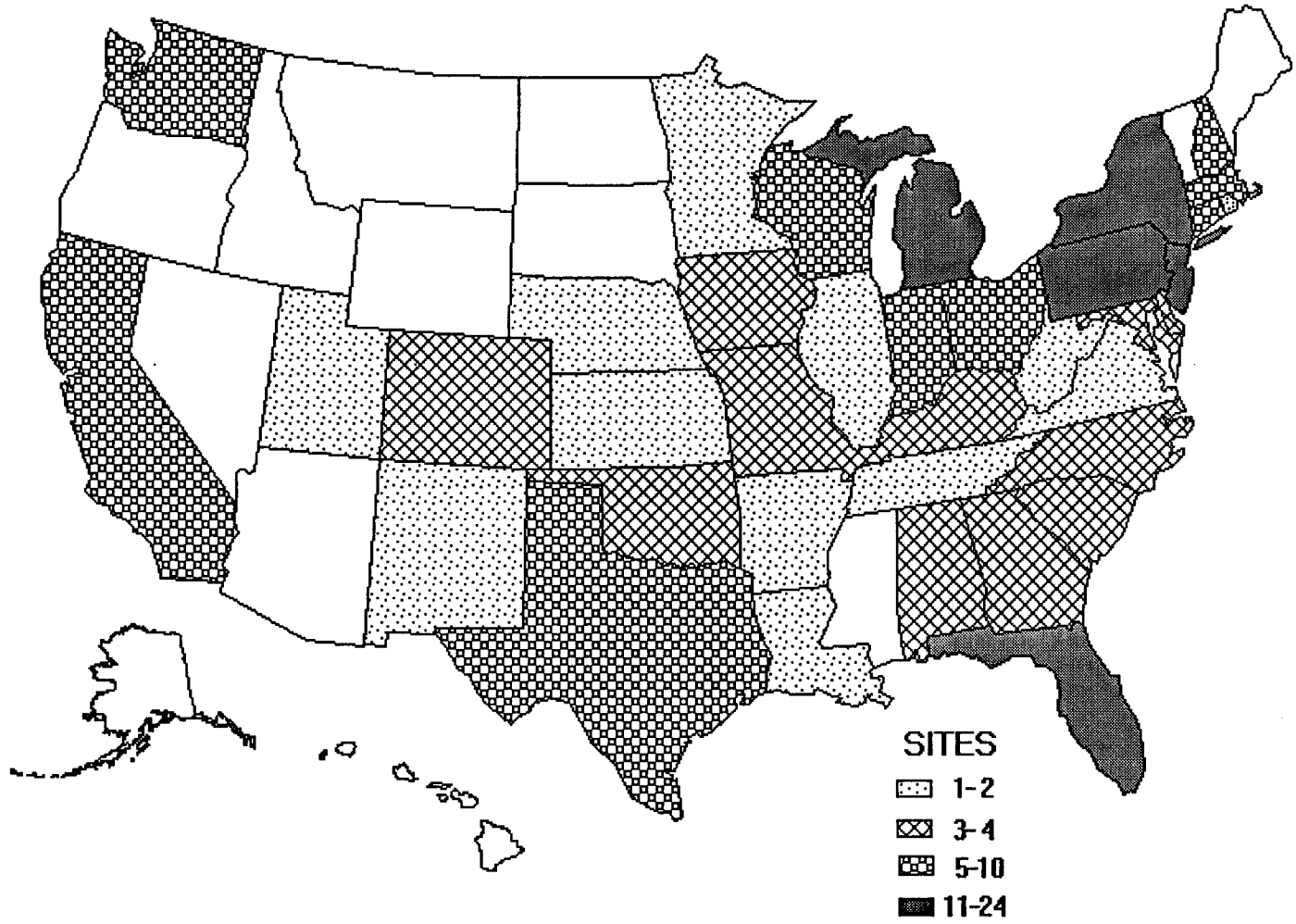
Inhalation of carbon disulfide in workplace air is generally the main route of human exposure to the compound, with skin exposure also important when the solvent is handled manually.

Carbon disulfide has been identified in at least 200 of the 1,430 current or former EPA National Priorities List (NPL) hazardous waste sites (HazDat 1996). It is not known how many of the 1,430 sites have been evaluated for carbon disulfide. As more sites are evaluated by EPA, this number may change. The frequency of these sites within the United States can be seen in Figure 5-1. Of these sites, 1 is located in the Commonwealth of Puerto Rico.

5.2 RELEASES TO THE ENVIRONMENT

According to the Superfund Amendments and Reauthorization Act (SARA), Section 313, Toxics Release Inventory (TRI93 1995), an estimated total of at least 93, 308, 704 pounds of carbon disulfide were released to the environment from manufacturing and processing facilities in the United States in 1993 (see Table 5-l). This total includes an estimated 2,805 pounds that were released through underground injection. The TRI data must be viewed with caution, however, since only certain types of facilities are required to report.

FIGURE 5-1 FREQUENCY OF NPL SITES WITH CARBON DISULFIDE CONTAMINATIONS



*Derived from HAZDAT 1996

Table 5-1. Releases to the Environment from Facilities That Manufacture or Process Carbon Disulfide

		Range of reported amounts released in pounds per year[a]						
State[b]	Number of facilities	Air	Water	Land	Underground injection	Total environment[c]	POTW transfer	Off-site waste transfer
AL	5	130-42610000	0-14000	0	0	130-42624000	0	0-120320
AR	2	589679-1113000	0-250	0	0	589679-1113250	0	0
AZ	2	10-1000	0	0	0	10-1000	0-5	0
CA	2	10-8272	0	0	0	10-8272	0	0
CT	2	11-2148	0	0	0	11-2148	2-4137	0
DE	1	0	0	3	0	3	0	0
GA	3	310-2240	0	0	0	310-2240	0-250	0
IL	5	0-3532872	0-250	0	0	0-3532872	0-130000	0-250
IN	3	250-2340000	0	0	0	250-2340000	0	0
KS	2	337706-948000	0	0	0	337706-948000	0	0-250
KY	1	3080	0	0	0	3080	170	51
LA	9	21-2719497	0	0	0-2800	21-2719497	0	0-10
MN	1	0	0	0	0	0	0	0
MO	3	0-644	0-1	0	0	0-645	0-4	0-382
MS	1	5	0	0	0	5	0	0
MT	1	3433	0	0	0	3433	0	0
NJ	2	167-25000	0-11	0	0	178-25000	0-1000	0-55830
NY	2	3748-544000	0	0	0	3758-544000	570-9300	0-3890
OH	4	427-317000	0	0	0-5	427-317000	0	0-2
OK	1	413200	0	0	0	413200	0	0
PA	1	70900	250	0	0	71150	0	510
SC	2	2-1000	0	0	0	2-1000	250	0
TN	5	1-22250000	0-18023	0	0	1-22250000	0-71000	0
TX	12	5-288660	0-250	0-5	0	5-288660	0-750	0-15737
VA	1	4900	50	0	0	49050	0	0

Table 5-1. Releases to the Environment from Facilities That Manufacture or Process Carbon Disulfide

		Range of reported amounts released in pounds per year[a]						
State[b]	Number of facilities	Air	Water	Land	Underground injection	Total environment[c]	POTW transfer	Off-site waste transfer
WA	1	10	0	0	0	10	0	0
WI	1	257450	0	0	0	257450	1260	130
WV	5	121-522074	0-788	0	0	121-522074	0	0-255

Source: TRI93 1995
[a] Data in TRI are maximum amounts released by each facility
[b] Post office state abbreviations used
[c] The sum of all releases of the chemical to air, land, water, and underground injection wells by a given facility

POTW = publicly owned treatment works

5.2.1 Air

There are several known natural sources of carbon disulfide, including wetlands (Hines et al. 1993), oceans (Chin and Davis 1993), and microbial activity in soils (Banwart and Bremner 1975; Kanda et al. 1995). The quantity of carbon disulfide emitted from such natural sources as volcanic and geothermal activity is not known but is thought to be small compared to anthropogenic sources, particularly combustion of fossil fuels and other carbonaceous material (Chin and Davis 1993). Certain crop plants and trees emit small amounts of carbon disulfide as well (Batten et al. 1995; Hartel and Reeder 1993).

Historically, carbon disulfide was used in the processing of rubber, but changing technology made the old practices outmoded. Nevertheless, the use of sulfur to crosslink rubber during the vulcanization process may account for the carbon disulfide released from ground tire treads in laboratory experiments (Pos and Berresheim 1993). Automotive tire wear has been suggested as a potential source of atmospheric carbon disulfide. Currently, the largest single use of carbon disulfide is in the viscose rayon industry. For every kg of viscose used, 20-30g of carbon disulfide are emitted (WHO 1979). The largest nonpoint source of man-made levels of carbon disulfide results from its use in the laboratory and as a fumigant and from the degradation of rubber products (EPA 1975b). Small amounts of carbon disulfide have also been detected in a landfill simulator (Vogt and Walsh 1985) and in the odoriferous emissions from a sewage treatment plant (Ruby et al. 1987).

Point sources of carbon disulfide include the biological degradation and incineration of wastes such as municipal refuse, sewage sludge, and industrial wastes (EPA 1975b). Monitoring of air over the North Atlantic Ocean found the highest levels of carbon disulfide off the New Jersey/New York coast, downwind of industrial pollution sources in the northeastern United States (Cooper and Saltzman 1993).

According to TRI93 (1995), an estimated total of 93,271,722 pounds of carbon disulfide, amounting to 99.9% of the total environmental release, was discharged to the air from manufacturing and processing facilities in the United States in 1993 (TRI93 1995) (see Table 5-l). The data listed in TRI should be used with caution since only certain types of facilities are required to report.

Carbon disulfide has been detected in the magmatic gas over volcanoes, during the aging of roasted coffee, during the pressure cooking of grain-water mixtures, as a volatile constituent in the vapor of burning cigarettes, and in the vapor space above liquid sulfur (EPA 1978a). Carbon disulfide has been measured in atmospheric samples collected during the major eruptions of Mount St. Helens. Low levels desorbed from volcanic ash were found to decrease with increasing distance from the volcanic activity (Rasmussen et al. 1982).

During analytical measurements of sulfur compounds at five wetland areas in Florida, carbon disulfide was often not detected while large amounts of dimethylsulfide were found (Cooper et al. 1987). However, low levels of carbon disulfide were consistently detected in samples collected from the same area using a slightly modified procedure (Hines et al. 1993). Based on their measurements and assumptions in the study of sulfur emissions from a North Carolina salt marsh, Aneja et al. (1980) estimated that carbon disulfide produced by marshes (0.022 g sulfur/m^2 per year) contributes less than 0.07% of biogenic sulfur and less than 8% to the stratospheric aerosol layer. DeMello et al. (1987) speculated that carbon disulfide generation from coastal areas in Florida was related to the concentration of organic matter in the sediment. Staubes et al. (1987) found that humic soils were stronger sources for biogenic sulfur than soils with lower organic content; however, a low humic content coupled with high moisture favors the production of carbon disulfide over dimethylsulfide.

In order to avoid the difficulties of naturally occurring variations in study conditions, Fall et al. (1988) studied the emission of sulfur gases from several plant/soil systems using a flux chamber. The study was designed so that emissions from soil could be separated from emissions from plants. Variable amounts of carbon disulfide were emitted from wheat. The effects of light and temperature were observed. Further work was proposed so that systematic investigation could accurately measure the contributions of a number of sulfur compounds under varying conditions.

5.2.2 Water

According to TRI, an estimated total of at least 34,169 pounds of carbon disulfide, amounting to 0.04% of the total environmental release, was discharged to water from manufacturing and processing facilities in the United States in 1993 (TRI93 1995) (see Table 5-l). In addition, an estimated total of 226,215 pounds, amounting to 0.24% of the total environmental release, was discharged to publicly

owned treatment works. The TRI data should be used with caution since only certain types of facilities are required to report.

Carbon disulfide is widely found in coastal and ocean waters and extensive study has been done to determine levels over the different types of water bodies. The measurements of Carroll (1985) show that the ocean appears to be a source of carbon disulfide, possibly via anaerobic microorganisms. Concentrations of less than 10 nmol/L have been found in a sulfide-rich lake in Spain (Simo et al. 1993). Carbon disulfide has also been detected in the vent fluids and sediment surface waters of undersea hydrothermal sites (Marchand et al. 1994).

Carbon disulfide was found at a concentration of 25 μg/L in groundwater samples collected from only 1 of 19 municipal solid waste landfills examined by Battista and Connelly (1989).

The South Carolina Department of Health (1986) found unspecified levels of carbon disulfide in groundwater samples collected from 1 of 11 wells constructed in a surficial aquifer near a recycling and disposal company that had been storing chemicals.

In a study of 63 industrial effluents collected from a wide range of chemical manufacturers from across the United States, carbon disulfide was found in six of the effluents at concentrations less than 10 pg/L and in two of the effluents at l0-100 μg/L (EPA 1979). Analysis of influent and effluent samples from a waste water treatment plant in Tokyo showed carbon disulfide to be the least prevalent among five sulfur-containing odorous compounds for which testing was done (Hwang et al. 1995).

5.2.3 Soil

According to TRI, an estimated total of at least 8 pounds of carbon disulfide was discharged to soils from manufacturing and processing facilities in the United States in 1993 (TR193 1995) (see Table 5-l). The TRI data should be used with caution since only certain types of facilities are required to report.

Little information was found regarding releases of carbon disulfide to soils. Fain et al. (1987) reported 0.9 mg/L carbon disulfide (dry weight basis) in a typical refinery oily waste applied to a land treatment unit.

5.3 ENVIRONMENTAL FATE

5.3.1 Transport and Partitioning

Releases of carbon disulfide to the environment as a result of industrial activity are expected to be primarily to the atmosphere. Any carbon disulfide released to surface waters in effluent streams is expected to partition rapidly to the atmosphere as a result of the high ratio of vapor pressure to the solubility (Henry's law constant = 1.01×10^{-2} atm • m^3/mol) of the compound. Hydrolysis is not a significant removal mechanism since the evaporation half-life from a saturated solution is estimated to be 11 minutes (EPA 1978a).

Although no information was found evaluating the partitioning of carbon disulfide from water onto sediments, it is not expected to be removed significantly from the aquatic phase through adsorption. The low K_{oc} value, calculated from water solubility data, is 54 (EPA 1986b), indicates high soil mobility, but it probably will be less mobile in soils of high organic content.

Although Roy and Griffin (1985) did not conduct adsorption studies, they classified carbon disulfide as a mobile solvent exhibiting a low tendency to be retained by soils. Carbon disulfide released to soils in spills should rapidly volatilize to the atmosphere, but a portion of the compound remaining on soil surfaces could be available for transport into groundwater since it does not have much affinity for soil particles. Farwell et al. (1979) indicated that carbon disulfide volatilizes from a variety of soils, although rates were not provided.

No experimental data on biomagnification were found in the available literature. Estimated bioconcentration factor (BCF) values (equal to 2.94×10^3) were calculated from solubility and K_{ow}, (log K_{ow} is 2.16) data. The calculated values, 6.8 and 25.8 respectively for solubility and K_{ow} data, indicate that carbon disulfide will not significantly bioaccumulate in aquatic organisms (EPA 1986b).

5.3.2 Transformation and Degradation

5.3.2.1 Air

Carbon disulfide reacts with hydroxyl radicals in the troposphere to produce carbonyl sulfide (Cox and Sheppard 1980). The lifetime of carbon disulfide in the troposphere, assuming a reaction rate constant of $4.3x10^{-13}$ cm^3 $molecule^{-3}$, is =73 days (uncertain); other estimates (assuming different reaction rate constants) range from less than 1 week to more than 10 weeks (Cox and Sheppard 1980; EPA 1978a; Wine et al. 1981).

The photo-oxidation products of carbon disulfide in the laboratory were identified as carbon monoxide, carbonyl sulfide, sulfur dioxide, and a polymer that adhered to the sides of the reaction vessel (Heicklen et al. 1971). Although carbon disulfide absorbs light at wavelengths between 280 and 350 nm, dissociation does not occur under environmental conditions because of low molar absorptivity (Atkinson et al. 1978; Wood and Heicklen 1971) and direct photolysis of carbon disulfide in the atmosphere does not appear to be significant. EPA (1978a) stated that the information available indicated that carbon disulfide is relatively persistent in the atmosphere. For the atmospheric oxidation of carbon disulfide to sulfur dioxide, car-bony1 sulfide, and carbon monoxide, the half-life was estimated to be about 12 days.

According to Wine et al. (1981), electronically excited carbon disulfide is rapidly produced in the troposphere from absorption of solar photons. This excited carbon disulfide reacts with oxygen on a time scale of l-2 weeks to yield car-bony1 sulfide, the predominant sulfur-containing compound in the troposphere.

The lifetime of carbon disulfide in the atmosphere has been estimated to be 12 days, too short a time to reach the stratosphere. Removal was suggested to occur by a hydroxyl radical reaction, or an oxygen atom reaction but not by dissociation (Khalil and Rasmussen 1984).

Based on the estimates of a lifetime in the troposphere for carbon disulfide on the order of weeks and the troposphere to stratosphere turnover time on the order of years, very little tropospheric carbon disulfide is expected to be transported to the stratosphere (EPA 1986b).

5.3.2.2 Water

Carbon disulfide is stable to hydrolysis in the pH region of environmental concern (pH 4-10). At pH 13, carbon disulfide has a hydrolysis half-life at of about 1 hour at 25°C; by extrapolation, at pH 9, carbon disulfide has a half-life of 1.1 years (EPA 1978a). In oxygenated seawater, carbon disulfide was found to be stable for over 10 days (Lovelock 1974). The volatilization half-life from a saturated water solution has been estimated to be 11 minutes (EPA 1978a). The compound apparently does not undergo biodegradation at rates that are competitive with its volatilization from surface waters.

5.3.2.3 Sediment and Soil

No data were found in the available literature on the biodegradation of carbon disulfide in soil. However, since the chemical is rapidly volatilized (high Henry's law constant) and probably highly mobile in soil (low K_{oc}), it is unlikely that it remains in the soil long enough to be significantly biodegraded.

Microbial degradation of large amounts of carbon disulfide in soil would not be expected to be significant since this compound is a soil disinfectant and toxic to bacteria. Hydrolysis of carbon disulfide on wet soil surfaces is also unlikely (EPA 1986b). Oxidation of carbon disulfide by a Thiobacillus species isolated from soil has been observed (Plas et al. 1993).

5.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

5.4.1 Air

Carbon disulfide was detected at 41 parts per trillion (ppt) in 61 rural samples and at 65 ppt in 88 urban/suburban air samples collected by Brodzinsky and Singh (1983). Carroll (1985) sampled air in the vicinity of San Juan, Puerto Rico; Albany, New York; and Wallops Island, Virginia. Carbon disulfide showed considerable spatial variability and a correlation with cloud activity. It was observed that the ocean appears to be a source of carbon disulfide. The air at Wallops Island coming in from the ocean had levels of 30 ppt. Air samples taken at Sapelo Island, Georgia, revealed carbon disulfide levels of about 380 ppt above a saltwater marsh, about 100 ppt above a freshwater marsh, and

negligible amounts above a swamp (Berresheim 1993). Levels of about 12 parts per trillion by volume (pptv) were measured near the island of Fiji in the southern Pacific Ocean (Thornton and Bandy 1993), while levels of 5.8 and 4.2 pptv were measured in marine and continental air, respectively, over the North Atlantic (Cooper and Saltzman 1993). Tropospheric carbon disulfide levels above the Atlantic Ocean were 0.5-5 pptv and l-30 pptv in areas influenced by anthropogenic or marsh emissions, respectively (Bandy et al. 1993b).

Air in the Philadelphia, Pennsylvania, area that had been influenced by anthropogenic sources was measured at 65-339 pptv carbon disulfide by Maroulis and Bandy (1980). Daily mean carbon disulfide levels in Philadelphia were highly dependent on wind direction.

Air measurements in the vicinity of a municipal landfill showed downwind concentrations three to four times higher than upwind (background) levels (Marquardt 1987). The maximum concentration of carbon disulfide measured in samples from eight solid waste composting facilities was 150 $\mu g/m^3$ (0.05 ppm) (Eitzer 1995).

Breath and air collected at sites in and around New York City were measured for carbon disulfide by Phillips (1992). The group tested consisted of four types (male smokers, male nonsmokers, female smokers, and female nonsmokers) with no significant divergence observed among any of the different group types (see Table 5-2). Carbon disulfide was detected in all air and breath samples taken from both indoor air and outdoor locations (see Table 5-3). A general population survey in Italy found low levels of carbon disulfide in the blood of all 208 subjects tested and in the urine of all 1,256 samples taken (Brugnone et al. 1994).

5.4.2 Water

It has been suggested that anaerobic conditions on the ocean floor result in formation of carbon disulfide (Lovelock 1974). Measurements made off the coast of Ireland showed carbon disulfide present at every location, with the highest concentrations in stagnant bays.

In a preliminary report to Congress, EPA (1975a) noted that carbon disulfide had been found (detection levels unspecified) in the drinking water of two of five U.S. cities studied. Krill and

TABLE 5-2. Carbon Disulfide Levels in Breath and Air[a]

	Number of observations	Mean age (years)	Standard deviation	Mean carbon disulfide concentration (pmol/L)[b,c]	Standard deviation
Male smokers	8	35.7	4.82	4.80	3.19
Male nonsmokers	12	32.9	6.44	6.32	5.24
Female smokers	12	36.8	9.34	5.79	3.49
Female nonsmokers	10	34.5	9.20	3.67	2.79
All volunteers	42	34.9	7.68	5.25	3.89
Room air	9			8.26	5.58
Outdoor air	6			3.92	0.63

[a] Adapted from Phillips (1992)
[b] No significant differences observed between any of the groups
[c] 1 pmol/L = 0.025 ppb

TABLE 5-3. Carbon Disulfide in Outdoor Air at Sites in and around New York City[a]

Collection site	Carbon disulfide (pmol/L)[b]
Empire State Building (observation deck)	4.01
Times Square	3.98
Bayley Seton Hospital, Staten Island (roof)	4.61
New Jersey Turnpike (Newark Airport toll plaza)	4.13
Central Park (West 85th Street entrance)	4.08
Brighton Beach boardwalk, Brooklyn	2.72

[a] Adapted from Phillips (1992)
[b] 1 pmol/L = 0.025 ppb

Sonzogni (1986) reported on an analytical survey of municipal and private groundwater sources in Wisconsin in which carbon disulfide was found in two private wells at concentrations lower than 830 μg/L (the published health advisory level). Carbon disulfide was also found in borings on the site of a landfill in Jacksonville, Florida (EPA 1986c). A survey of groundwater samples in northeastern Florida found no detectable levels of carbon disulfide in underground storage tank system sites and only one landfill site with a measurable level of 21.5 μg/L (Pawlowicz 1993).

Carbon disulfide was measured in Lake Ontario (Kaiser et al. 1983). Mapping of the results showed that levels were high in Toronto Harbor where textile, glass, metal, and plastic manufacturing plants are adjacent to the lake (the highest level detected was 3.9 mg/L). Carbon disulfide was also found in the Niagara River at 25 μg/L. Kaiser and Comba (1983) measured levels as high as 25 μg/L in Thompson Creek near a chemical plant discharge (Welland River watershed in Ontario).

5.4.3 Sediment and Soil

Very little information is available on the levels of carbon disulfide in soils. EPA (1986d) reported finding carbon disulfide at low pg/kg levels in the soil where paint sludges had been dumped on the ground in a gravel pit in Michigan.

5.4.4 Other Media

An analysis of fumigated grains for carbon disulfide showed levels ranging from 0.8 to 2.2 ppm decreasing with time after fumigation, as would be expected from the volatility of carbon disulfide (McMahon 1971). Lovegren et al. (1979) detected 2-3 ppb carbon disulfide in dried legumes. In a study of fumigant residues in foods, Daft (1987) reported finding carbon disulfide residues in samples of pickling spice, mustard seed, and other grain foods. Heikes (1987) examined table-ready foods and found carbon disulfide in plain granola, and Daft (1988a, 1988b) found carbon disulfide in raw onions, raw radishes, corn chips, and oat cereal. The pesticide sodium tetrathiocarbonate may be converted to carbon disulfide after application to fruit crops, and EPA has set a tolerance level of 0.1 ppm carbon disulfide from this source for grapefruit, grapes, lemons, and oranges (EPA 1993, 1995d).
The levels of carbon disulfide and other sulfur species emitted in oil shale off-gases were measured (Sklarew et al. 1984). The concentration of carbon disulfide ranged from 8 to 28 ppm. They

concluded that strategies for the use of oil shale as an alternative fuel must include abatement strategies.

Carbon disulfide was also found in the incinerator ash of hazardous waste incinerators (Wolbach et al. 1987). This finding was not anticipated because of the volatility of carbon disulfide. In addition, Kallonen et al. (1985) identified carbon disulfide at low levels (less than 1 μg/L) in fire gas emissions from the combustion of wool. Exposures at this level do not usually result in significant body concentration of carbon disulfide.

5.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

Exposure to carbon disulfide is primarily confined to occupational situations. Inhalation is the principal route of exposure for humans; absorption through the skin is a much less important route than inhalation, and other routes are negligible (WHO 1979). Very little information is available concerning exposure to carbon disulfide outside the workplace or the effects on the general population. Teisinger and Soucek (1949) reported that, in spite of considerable variation among individuals, absorption seems to be proportional to the concentration of carbon disulfide in inhaled air. Assuming an inhalation intake of 20 m^3 air/day, and concentrations in urban and rural air of 65 ppt and 41 ppt, respectively, the average daily intake of carbon disulfide via inhalation is 4.1×10^{-3} mg in urban areas and 2.6×10^{-3} mg in rural areas.

From the limited data available, it appears that individuals living close to workplaces where carbon disulfide is used can be exposed to high enough concentrations to result in measurable uptake (WHO 1979). However, in a study that compared 70 children living 400 meters from a factory discharging carbon disulfide into the atmosphere with a control group of 30 children living 15 km from the factory, physical examinations did not show any health disorders in the exposed group even though urine concentrations of carbon disulfide indicated increased uptake compared with the controls (Helasova 1969).

Airborne concentrations of carbon disulfide in a domestic rayon plant were reported to range from 10 to 15 ppm (NIOSH 1977).

During a 1991 study of a Belgian viscose rayon factory, workers from various departments in the company were monitored with charcoal tubes for carbon disulfide (Vanhoorne et al. 1991). The geometric mean for carbon disulfide ranged from 3.67 mg/m^3 (1.17 ppm) to 147.23 mg/m^3 (47.11 ppm) (Vanhoorne et al. 1991). Eighty-nine percent of the job classifications studied exceeded the present TLV for carbon disulfide of 31 mg/m^3 (10 ppm) (Vanhoorne et al. 1991). Typically, workers are simultaneously exposed to both carbon disulfide (4-l 12 mg/m^3) and hydrogen sulfide (0.2-8.9 mg/m^3) (Vanhoorne et al. 1995). Various studies throughout the world (see Table 5-4) indicate that there has been a recent decline in personal exposure of workers in Western countries to carbon disulfide, but in the Far East and Eastern Europe (Rigterink 1988) exposure to carbon disulfide of workers is still substantial.

According to the National Occupational Exposure Survey (NOES) conducted by NIOSH from 1980 to 1983, an estimated 44,438 workers, including 4,881 women, were potentially exposed to carbon disulfide in the workplace in 1980. The NOES database does not contain any information about the concentration levels to which these workers may have been exposed or the frequency and duration of the exposure (NIOSH 1989).

Carbon disulfide was found at unspecified levels in all eight samples of human breast milk tested during a study conducted in New Jersey, Pennsylvania, and Louisiana (Pellizzari et al. 1982). Cai and Bao (1981) measured the levels of carbon disulfide in the milk of mothers employed in rayon factories at 68-123 μg/L and found that carbon disulfide was still present in the milk after a month or more away from the factory.

5.6 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Human exposure to carbon disulfide is expected to be highest among certain occupational groups (e.g., rayon plant workers). In addition, members of the general population living in the vicinity of industrial point emission sources are exposed to higher than background levels of carbon disulfide. The compound has been detected in both ambient air and water in low concentrations, with somewhat higher concentrations in localized areas around industrial and disposal sites. No information was found regarding the number of people potentially exposed in the vicinity of hazardous waste sites. However, since carbon disulfide has been found near hazardous waste sites, people living near them may be exposed to higher than background levels.

TABLE 5-4. Personal Carbon Disulfide Exposure in Some References in the Viscose Study[a]

Country	Type of viscose production[b]	n[c]	Average carbon disulfide (mg/m^3)[d] Minimum[e]	Maximum[f]	Method[g]	Reference
United States	a	25	0.21	15.40	A	Fajen et al. 1981
China	a	8	0.08	15.55	B	Sugimoto et al. 1982
Sweden	a	7	1.40	2.40	A	Westberg et al. 1984
France	a	23	<0.30	39.90	A	Cicolella and Vincent 1986
	b	14	2.10	35.40	A	
	c	7	0.60	68.70	A	
	d	29	9.60	33.30	A	
	e	26	<0.30	29.10	A	
The Netherlands	b	11	6.20	50.60	B	Verwijst 1988
Belgium	c	17	3.67	147.23	A	Vanhoorne et al. 1991
			4.00			Vanhoorne et al. 1995

[a]Adapted from Vanhoorne et al 1991
[b]Type of production: (a) staple fibre; (b) yarn, continuous; (c) yarn, discontinuous; (d) sponges; (e) sausage casings
[c]Number of jobs studied
[d]Milligram per cubic meter of air; 1 mg/m^3 = 0.32 ppm
[e]Jobs with the lowest average exposure
[f]Jobs with the highest average exposure
[g]Methods: (a) charcoal tubes-personal sampling pump; (b) diffusive sampling

Of particular concern would be a worker with occupational exposure to carbon disulfide who lived close enough to the plant to be exposed to elevated levels at home as well, particularly if that worker was in poor health from other causes (e.g., smoking, neurological problems, or heart disease).

5.7 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of carbon disulfide is available. Where adequate information is not available, ATSDR, in conjunction with the NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of carbon disulfide.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

5.7.1 Identification of Data Needs

Physical and Chemical Properties. The physical and chemical properties of carbon disulfide are sufficiently well defined to allow an assessment of its environmental fate (EPA 1995h; Flick 1985; HSDB 1995; MCA 1968; NFPA 1986; NIOSH 1984b; RTECS 1995; Sax and Lewis 1987; Timmerman 1978; Verschueren 1983; Weast 1989; Weiss 1980; Windholz 1983; Worthing 1987). Therefore, no data needs have been identified at this time.

Production, Import/Export, Use, Release, and Disposal. Little specific information is available on the levels of carbon disulfide emitted from industrial sources. Most of the literature concentrates on the viscose rayon industry (EPA 1975b; Battista and Connelly 1989; Carroll 1985; CIS 1989; Cooper et al. 1987; DeMello et al. 1987; EPA 1979, 1978a; Fain et al. 1987; Rasmussen et al. 1982; Ruby et al. 1987; Sax and Lewis 1987; South Carolina DOH 1986; Spencer 1982; SRI 1989; Staubes et al. 1987; Timmerman 1978; TRI93 1995; View 1989; Vogt and Walsh 1985; WHO 1979,

1981; Windholz 1983; Worthing 1987) and natural sources (Timmerman 1978; Who 1979). Although future production levels of carbon disulfide are uncertain because of a long-term decline in the demand for viscose rayon and cellophane and restrictions on the use of fluorocarbon propellants, it is expected that the demand for this chemical in many other specialty areas will continue at relatively stable levels (Timmerman et al. 1978). Carbon disulfide is used primarily in industry. Releases from industrial processes are almost exclusively to the atmosphere. Sources and releases of carbon disulfide to the immediate environment should be identified and quantified, and additional information is needed on the disposal of carbon disulfide. Current disposal methods include liquid injection incineration, rotary kiln incineration, fluidized bed incineration, and air stripping (HSDB 1995), however, data on the efficiency of these methods are lacking. This information will be useful in identifying the media of concern for human exposure and populations at risk of adverse health effects from exposure to carbon disulfide.

According to the Emergency Planning and Community Right-to-Know Act of 1986, 42 U.S.C. Section 11023, industries are required to submit substance release and off-site transfer information to the EPA. The TRI, which contains this information for 1988, became available in May of 1990. This database is updated yearly.

Environmental Fate. Releases of carbon disulfide to the environment as a result of industrial activity are expected to be primarily to the atmosphere. Carbon disulfide volatilizes from a variety of soils (Farwell et al. 1979). Carbon disulfide reacts with hydroxyl radicals in the troposphere to produce carbonyl sulfide (Cox and Sheppard 1980). Further oxidation would produce carbon disulfide, a major contributor to the greenhouse effect (Cox and Sheppard 1980). The lifetime of carbon disulfide in the troposphere is ≈73 days (Cox and Sheppard 1980). Carbon disulfide is stable to hydrolysis in the pH region of environmental concern (pH 4-10), with a hydrolysis half-life at pH 13 of about 1 year (EPA 1976). No data are available concerning the biodegradation of carbon disulfide in soil. Concerted efforts should be made to measure the spatial and temporal variations in the atmospheric levels of carbon disulfide in the vicinity of specific point or nonpoint sources. Although volatilization is the primary fate of carbon disulfide released to the environment (Farwell et al. 1979; Roy and Griffin 1985), data on the partitioning of carbon disulfide from water onto sediments and on the hydrolysis rate of carbon disulfide in surface and groundwater could be useful in determining the persistence of low levels of the compound in the environment. Additional information on the transport and transformation of carbon disulfide in soils, particularly on biotransformation, would also be useful.

Bioavailability from Environmental Media. Carbon disulfide is absorbed following inhalation of contaminated ambient air (Soucek 1957; Teisinger and Soucek 1949) and from dermal contact with contaminated soils or water (Helasova 1969; NIOSH 1989). The average daily intake of carbon disulfide via inhalation has been estimated to be 4.1×10^{-3} mg in urban areas and 2.6×10^{-3} mg in rural areas. Data are lacking on the bioavailability of carbon disulfide following ingestion of contaminated soils and groundwater or foods grown with contaminated water. This information would be useful in determining the importance of these routes of exposure.

Food Chain Bioaccumulation. An estimated bioconcentration factor (BCF) of 2.94×10^{3} was calculated from solubility and K_{ow} data. Based on these data, carbon disulfide does not significantly bioaccumulate in aquatic organisms. Although it is generally accepted that carbon disulfide does not bioaccumulate because of its rapid metabolism (EPA 1986b), the data are insufficient to determine the bioconcentration and biomagnification of carbon disulfide at various levels in the food chain. This information would be particularly useful in determining the risks associated with the generally low levels of carbon disulfide in environmental media.

Exposure Levels in Environmental Media. The monitoring data available for carbon disulfide are too limited to allow adequate characterization of potential human exposure to the compound. Studies of background levels in air have been conducted (Bandy et al. 1993b; Brodzinsky and Singh 1983; Carroll 1985; Cooper and Saltzman 1993; Maroulis and Bandy 1980; Marquardt 1987), but site specific concentration data for ambient air, drinking water, and biota, particularly at hazardous waste sites, are lacking. These data would be helpful in estimating the exposure of the general population as well as those living near hazardous waste sites. The sites with highest concentrations of carbon disulfide need to be determined. In addition, estimates of human intake from various media would be helpful in assessing human exposure for carbon disulfide for populations living near hazardous waste sites.

Reliable and current monitoring data for the levels of carbon disulfide in contaminated media at hazardous waste sites are needed so that the information obtained on levels of carbon disulfide in the environment can be used in combination with the known body burden of carbon disulfide to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

Exposure Levels in Humans. Carbon disulfide can be detected in exhaled breath, blood, urine, and milk, and metabolites can be detected in urine, exhaled air, and blood (ACGIH 1986; Baselt 1980; Cai and Bao 1981; Campbell et al. 1985; Cox et al. 1992; Djuric 1967; Helasova 1969; Lieben 1974; McKee et al. 1943; NIOSH 1989; Pellizzari et al. 1982; Teisinger and Soucek 1949; WHO 1979). However, because of the rapid metabolism and elimination of carbon disulfide, these fluid and breath levels do not correlate well with environmental levels, except for the urinary marker, 2-thiothiazolidine-4-carboxylic acid. In addition, the interaction of carbon disulfide with other potential confounders may affect the reliability of urinary metabolites as biomarkers of exposure. Biomarkers may therefore be of limited utility in the quantitative assessment of human exposure to carbon disulfide at hazardous waste sites; however, biomarkers may be useful in qualitatively establishing that possible exposure has occurred.

Additional information on biological monitoring is necessary for assessing the need to conduct health studies on general populations and on those populations living near hazardous waste sites.

Exposure Registries. No exposure registries for carbon disulfide were located. This substance is not currently one of the compounds for which a subregistry has been established in the National Exposure Registry. The substance will be considered in the future when chemical selection is made for subregistries to be established. The information that is amassed in the National Exposure Registry facilitates the epidemiological research needed to assess adverse health outcomes that may be related to exposure to this compound.

5.7.2 On-going Studies

As part of the Third National Health and Nutrition Evaluation Survey (NHANES III), the Environmental Health Laboratory Sciences Division of the National Center for Environmental Health, Centers for Disease Control and Prevention, will analyze human blood samples for carbon disulfide and other volatile organic compounds. These data will give an indication of the frequency of occurrence and background levels of these compounds in the general population.

The data generated as a result of the remedial investigation/feasibility studies of the 200 NPL sites known to be contaminated with carbon disulfide should add to the current knowledge regarding the environmental transport and fate of the compound.

No long-term research projects or other on-going studies of occupational or general population exposures were identified.

6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, and/or measuring, and/or monitoring carbon disulfide, its metabolites, and other biomarkers of exposure and effect to carbon disulfide. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

6.1 BIOLOGICAL SAMPLES

A limited number of analytical techniques have been used for measuring carbon disulfide and its metabolites in breath (expired air) and biological fluids of humans and animals. These include gas chromatography (GC) and high resolution gas chromatography (HRGC) equipped with an appropriate detector, high-performance liquid chromatography (HPLC), spectrophotometry, thin-layer chromatography (TLC), combined gas chromatography and mass spectroscopy (GC/MS), and iodineazide tests (see Table 6-l).

GC/MS was used in a 1992 study (Phillips 1992) to detect carbon disulfide in human breath and environmental air with a detection sensitivity capable of 7.61×10^{-2} μg/m^3 (2.44×10^{-2} ppb). This highly sensitive technique can be rapidly accomplished by capturing the air sample on solid sorbent like molecular sieves to be later thermally desorbed in the laboratory with subsequent chromatography (Phillips 1992).

Gas chromatography equipped with a flame ionization detector (FID) and quadrupole MS have been employed for measuring carbon disulfide concentrations in the breath of workers following exposure to carbon disulfide (Campbell et al. 1985; Wells and Koves 1974). The MS technique is rapid and requires no sample preparation (Campbell et al. 1985). A detection limit of 1.6 ppb (5 μg/m^3) of

TABLE 6-1. Analytical Methods for Determining Carbon Disulfide and Its Metabolites in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Breath (expired air)	Exhale into a respiratory MS	Quadrupole MS	5 μg/m^3	NR	Campbell et al. 1985
	Bubble sample through a solution of diethylamine in acetone and methyl iodide; acidify and extract sample with butyl chloride	GC/FID	No data	NR	Wells and Koves 1974
	Capture in a sorbent trap containing graphitized carbon and molecular sieve; thermally desorb; concentrate	GC/MS	76 ng/m^3	NR	Phillips 1992
Blood	Acidify blood sample and introduce into a headspace analyzer	GC/FPD	15 μg/L	NR	Campbell et al. 1985
	Add acid and Viles' reagent to sample and warm; purge-and-trap and measure cupric diethyldithiocarbamate at 430 nm	Spectrophotometer	ppm levels	NR	Lam and DiStefano 1982, 1983

TABLE 6-1. Analytical Methods for Determining Carbon Disulfide and Its Metabolites in Biological Materials (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
	Add internal standard (methyl ethyl ketone in water); agitate; transport from headspace and strip using a cyrogenic trap containing Tenax; thermally desorb	GC/MS	ng/L levels	NR	Brugnone et al. 1993, 1994; Perbellini et al. 1994
Urine (metabolites)	Add urine sample to a solution containing sodium azide, iodine, and potassium iodide	Iodine-azide test	16 ppm	NR	NIOSH 1977
			No data	NR	Baselt 1980
Urine (TTCA)	Acidify urine sample, extract with ether and evaporate; dissolve residue in methanol and analyze	HPLC	82 ng/L	NR	Campbell et al. 1985
	Add internal standard (300 mg sodium sulfate, 100 uL 6M HCl); extract with diethyl ether; dry and resuspend in phosphoric acid	HPLC	25 ng/L	>90%	Lee et al. 1995
	Direct injection of urine after dilution	HPLC	100 ng/mL	95–97%	Simon and Nicot 1993

TABLE 6-1. Analytical Methods for Determining Carbon Disulfide and Its Metabolites in Biological Materials (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine (MTZ and thiocarbamide)	Extract sample with ethylacetate and apply extract on TLC plate	GC/MS	No data	NR	Pergal et al. 1972a, 1972b
Milk	Warm sample; purge with helium and trap on Tenax® cartridge; thermal desorption with helium	HRGC/EIMS	NR	NR	Pellizzari et al. 1982
Milk	Warm sample in waterbath at 60°C; purge by aeration and trap in bubbler containing diethylamine-copper solution; make colorimetric determination at 420 nm	Spectrophotometry	μg levels	90%	Cai and Bao 1981

GC/FID = gas chromatography/flame ionization detector; GC/FPD = gas chromatography/flame photometric detector; GC/MS = gas chromatography/mass spectrometry; HPLC = high-performance liquid chromatography; HRGC/EIMS = high-resolution gas chromatography/electron impact mass spectrometry; MS = mass spectrometry; MTZ = 2-mercapto-2-thiazolinone-5; NR = not reported; TLC = thin-layer chromatography; TTCA = 2-thiothiazolidine-4-carboxylic acid

carbon disulfide in air was achieved using this technique. The FID technique is insensitive. Flame photometric, photoionization (11.6 eV) and electron capture detectors, or elemental detectors are sufficiently sensitive to ppb/ppm concentrations.

A spectrophotometric technique has been used for quantifying μg/rnL levels of free and acid-labile (chemically bound) carbon disulfide in the blood of rats (Lam and DiStefano 1982, 1983). This technique is based on measuring the absorbance at 430 nm of a yellow cupric diethyldithiocarbamate complex that is formed by reacting carbon disulfide in blood with Viles' reagent in the presence of acid and heat. A headspace sampler connected to GC equipped with a sulfur-specific flame photometric detector (FPD) has been developed for measuring low levels of free and acid-labile carbon disulfide in the blood of shift workers exposed to carbon disulfide (Campbell et al. 1985). A detection limit of 15.2 μg of carbon disulfide/L of blood was achieved. Concentrations of free and acid-labile carbon disulfide have also been determined by GS/MS (Brugnone et al. 1993, 1994; Perbellini et al. 1994).

It is also possible to determine the level of metabolites in urine. Urinary 2-thiothiazolidine-4-carboxylic acid is the best available indicator to assess the degree of occupational exposure to carbon disulfide (ACGIH 1994; Theinpont et al. 1990). Theinpont et al. (1990) described the isolation of this compound from urine prior to reverse phase high-performance liquid chromatography. It is based on liquid-liquid extraction with methyl tertbutyl ether, followed by affinity chromatography on organomercurial agarose gel. The detection limit of the procedure was 50 μg of carbon disulfide/L of urine (Theinpont et al. 1990).

The more common method of determining the level of metabolites in urine is HPLC. HPLC has been employed for measuring 2-thiothiazolidine-4-carboxylic acid in the urine of shift-workers following exposure to carbon disulfide (Campbell et al. 1985; Lee et al. 1995; Simon and Nicot 1993; Van Doorn et al. 1985a, 1985b). This technique is sensitive, specific, and noninvasive. A detection limit of 25 μg of carbon disulfide/L of urine was obtained (Lee et al. 1995). The iodine-azide test has been used for the detection of metabolites of carbon disulfide in the urine of humans and animals following exposure to carbon disulfide (Baselt 1980; Djuric 1967; WHO 1979). This method is based on the measurement of the time to decolor iodine, as catalyzed by sulfur-containing metabolites of carbon disulfide most notably thiourea and dithiocarbamates. However, the iodine-azide test is nonspecific and has relatively poor sensitivity for measuring the metabolites of carbon disulfide in the urine

(Baselt 1980; Djuric 1967). TLC has also been employed to detect 2-mercapto-2-thiazolin-5-one and thiocarbamide (metabolites of carbon disulfide) in the urine of workers exposed to carbon disulfide (Pergal et al. 1972a, 1972b; WHO 1979). GC/MS is used to confirm 2-mercapto-2-thiazolin-5-one, thiocarbamide, and TTCA.

Carbon disulfide has also been detected in mother's milk using HRGC/MS and spectrophotometry (Cai and Bao 1981; Pellizzari et al. 1982). Sample preparation for HRGC/MS involves purging the sample with helium and then trapping the analyte on a Tenax cartridge, followed by thermal desorption (Pellizzari et al. 1982). Sensitivity, precision, and recovery were not reported. For the spectrophotometric technique, sample preparation involves purging by aeration and trapping in a bubbler containing diethylamine-copper solution for calorimetric determination at 420 nm (Cai and Bao 1981). Recovery (90%) was excellent and sensitivity is in the μg range. Precision was not reported.

6.2 ENVIRONMENTAL SAMPLES

High-resolution GC equipped with an appropriate detector is the most common analytical technique for measuring the concentrations of carbon disulfide in air and various foods (e.g., grains, grain-based foods, fruits, and beverages). The choice of a particular detector will depend on the nature of the sample matrix, the detection limit, and the cost of the analysis. Because volatile organic compounds in environmental samples may exist as complex mixtures or at very low concentrations, preconcentration of these samples prior to quantification is usually necessary (see Table 6-2 for details).

The primary method of analyzing carbon disulfide in air is by adsorption on an activated charcoal tube followed by solvent elution for subsequent quantification. GC equipped with either an electron capture detector (ECD), photo-ionization detector (PID), or FPD has been used for measuring carbon disulfide after elution from the solid phase. Detection limits of low ppm levels of carbon disulfide in the air sample were achieved with these techniques (McCammon et al. 1975; Peltonen 1989; Smith and Krause 1978; UK/HSE 1983). NIOSH has recommended GC/FPD (method 1600) for determining carbon disulfide in air. The range of quantification is 3-64 ppm for a 5-L air sample (NIOSH 1984b).

TABLE 6-2. Analytical Methods for Determining Carbon Disulfide in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Adsorb sample on charcoal tube and desorb with toluene	GC/ECD	1.5 μg/m^3	NR	Peltonen 1989
	Adsorb sample on charcoal tube and desorb with acetonitrile	GC/PID	9.5 μg/m^3	100%	Smith and Krause 1979
	Adsorb sample on charcoal tube and desorb with toluene	GC/FPD	200 mg/sample; working range 3–64 ppm for a 5-L air sample	NR	NIOSH 1984 (Method 1600)
	Desorb sample from charcoal tube and react with pyrrolidine; react the resultant dithiocarbamate with copper to form a chelate; extract chelate with isoamyl acetate	AAS	0.7 μg/m^3	70–101%	Kneebone and Freiser 1975
	Adsorb sample on charcoal tube and desorb with benzene	GC/FPD	Low μg/m^3 levels	94%	McCammon et al. 1975
	Cryogenic preconcentration of sample (catalytic fluorination after GC to form SF_b)	GC/ECD	6.25 ng/m^3	NR	Johnson and Bates 1993

TABLE 6-2. Analytical Methods for Determining Carbon Disulfide in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
	Prepare with isotopically labelled internal standard (ILS); cryogenic preconcentration	GC/MS	0.625 ng/m^3	NR	Bandy et al. 1985, 1993a
	Scrub and dry air sample; cryogenic preconcentration	GC/SCD 355	10 pg	NR	Ivey and Swan 1995
Water, soil, and solid waste	Purge-and-trap sample; thermally desorb	GC/MS	low ppb level (water, solid waste); low ppm level (soil)	NR	EPA 1984; Haile and Lopez-Avila 1984; Hewitt et al. 1991 (EPA Methods 624, 8015, and 8240)
Water, soil, oil, and fish tissue	Vacuum distillation; trap vapors in a cryotrap	GC/MS	0.008 mg/m^3 in air; 0.01 mg/m^3 in soil; 0.6 mg/m^3 in oil; 0.017 mg/m^3 in tissue	110% in air; 50% in soil; 98% in oil; 79% in tissue	Hiatt et al. 1994

TABLE 6-2. Analytical Methods for Determining Carbon Disulfide in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Various foods (e.g., grains, grain-based foods, fruits, and beverages)	Ground sample in solution containing isooctane, phosphoric acid, and acetone	GC/ECD, GC/HECD	ng/g levels	NR	Daft 1988a, 1988b
	Steam-distill sample in toluene	GC/ECD	ng/sample	40–50%	Bielorai and Alumot 1966
	Reflux sample in isooctane and acid	GC/ECD	Low μg/g levels	59–105%	Malone 1970
	Extract sample by soaking in acetone:water (5:1)	GC/ECD	0.2 ng/sample	98–99% recovery	Heuser and Scudamore 1968
	Extract sample with acetone:water (5:1)	GC/ECD	No data	NR	AOAC 1984
	Dilute with acetone; static headspace technique	GC/SCD 355	ug/L levels	NR	Nedjma and Maujean 1995

AAS = atomic absorption spectrophotometry; EPA = Environmental Protection Agency; GC/ECD = gas chromatography/electron capture detector; GC/FPD = gas chromatography/flame photometric detector; GC/HECD = gas chromatography/ Hall's electrolytic conductivity detector; GC/MS = gas chromatography/mass spectrometry; GC/PID = gas chromatography/photoionization detector; ILS = isotopically labelled standard; NR = not reported; SCD = Sievers chemiluminescence detector

Gas chromatography with ECD has also been used to detect carbon disulfide in air (Johnson and Bates 1993). In this system, carbon disulfide is separated by chromatography, converted to sulfur hexafluoride (SF_6), and then detected by ECD. The ECD detector is 10-100 times more sensitive than the FPD detector and requires smaller sample volumes (Johnson and Bates 1993).

A.R. Bandy, D.C. Thornton, and A.R. Driedger (Drexel University, Philadelphia) have developed a GC/MS technique with isotopically labelled internal standards capable of real-time measurement of carbon disulfide and other volatile sulfur compounds in the air with a detection limit of 0.2 ppt. This technique is approximately 10 times more sensitive than GC/FPD for measuring carbon disulfide in the air and has improved precision and accuracy (Bandy et al. 1993a).

A less commonly used technique for quantifying carbon disulfide in air is atomic absorption spectrophotometry (AAS) for copper in a copper chelate whose concentration is related directly to carbon disulfide concentration (Kneebone and Freiser 1975). Analysis of carbon disulfide in air for AAS quantification involves desorbing the carbon disulfide from an activated charcoal tube with isoamylacetate containing pyrolidine. The eluate is shaken with acidified copper sulfate solution to form a copper chelate, which is quantified by AAS (Kneebone and Freiser 1975). A detection limit of 0.7 μg of carbon disulfide/m^3 of air was obtained. Carbon disulfide is usually isolated from various foods by liquid-liquid extraction and steam distillation (AOAC 1984; Bielorai and Alumot 1966; Clower et al. 1986; Daft 1988a, 1988b; Heuser and Scudamore 1968; Malone 1970; McMahon 1971). GC equipped with ECD or the Hall electrolyte conductivity detector (HECD) is the method of choice for measuring ppb levels of carbon disulfide in foodstuffs (Bielorai and Alumot 1966; Daft 1988a, 1988b; Heuser and Scudamore 1968).

6.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of carbon disulfide is available. Where adequate information is not available, ATSDR, in conjunction with the NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of carbon disulfide.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. GC (equipped with either FID, FPD, or MS), HPLC, spectrophotometry, and the iodine-azide test have been used for measuring carbon disulfide and its metabolites in the breath, urine, blood, and milk of humans and animals (Baselt 1980; Cai and Bao 1981; Campbell et al. 1985; Djuric 1967; Lam and DiStefano 1982, 1983; Pellizzari et al. 1982; Pergal et al. 1972a, 1972b; Wells and Koves 1974; WHO 1979). GC and HPLC are sensitive enough to measure background levels in the population, as well as levels at which biological effects might occur. Analysis of the parent compound is complicated by the chemical's high volatility and short half-life in biological materials, making detection of urinary metabolites a more reliable approach. Sensitive and selective methods of measuring urinary metabolites of carbon disulfide exist, but further studies might be useful in correlating measured levels of metabolites with carbon disulfide exposure levels and levels at which biological effects might occur.

No specific biomarkers of effect have been exclusively associated with carbon disulfide exposure. Some biological parameters, e.g., decreased nerve conduction velocity and changes in lipid metabolism, have been tentatively linked to carbon disulfide exposure, but there are insufficient data with which to assess the analytical methods associated with measurement of these potential biomarkers. Further investigations into these potential biomarkers, in conjunction with improvements in their detection methods might aid in establishing reliable biomarkers of effect for carbon disulfide.

At present, no specific biomarkers of exposure or effect other than the parent compound or its metabolites are available for carbon disulfide. However, the covalent cross-linking of erythrocyte spectrin by carbon disulfide may serve as a potential biomarker (Valentine 1993). There are no data to indicate whether a biomarker, if available, would be preferred over chemical analysis for monitoring exposure to carbon disulfide.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. A GC/MS method that uses an isotopically labelled variant of the analyte as an internal standard has been developed (Bandy 1985, 1993b). It is an accurate technique for the determination of ppt levels of carbon disulfide in the atmosphere. GC in combination with an electron capture sulfur detector is also sensitive enough to measure ppt levels in the atmosphere. GC equipped with either ECD, HECD, FPD, or PID is also used for measuring low ppm to ppb levels of carbon disulfide in air (Kneebone and Freiser 1975; McCammon et al. 1975; Peltonen 1989; Smith and Krause 1978) and in various foodstuffs such as grains, fruits, and beverages (AOAC 1984; Bieloral and Alumot 1966; Clower et al. 1986; Daft 1988a, 1988b; Heuser and Scudamore 1968; Malone 1970; McMahon 1971; NIOSH 1985; UK/HSE 1983). GC/MS is the analytical method used to measure low ppm to ppb levels of volatile organic compounds (VOCs) in water, soil, and solid waste (DOD 1991; EPA 1984b, 1984c). The air and foodstuffs are the media of most concern for potential human exposure to carbon disulfide. GC techniques are sensitive for measuring background levels of carbon disulfide in these media and levels of carbon disulfide at which health effects might begin to occur. NIOSH has recommended GC/FPD as the method (Method 1600) for measuring low levels of carbon disulfide in air. GC equipped with either ECD or HECD is the method of choice for measuring ppb levels of carbon disulfide in various foodstuffs. No additional analytical methods for measuring carbon disulfide in these environmental media appear to be necessary at this time. Although carbon disulfide was not one of the analytes mentioned in EPA methods for measuring VOCs in water, soil, and solid waste, the GC/MS method would be appropriate for measuring carbon disulfide in these media since it is a volatile compound.

6.3.2 On-going Studies

The Environmental Health Laboratory Sciences Division of the National Center for Environmental Health, Centers for Disease Control and Prevention, is developing methods for the analysis of carbon disulfide and other volatile organic compounds in blood. These methods use purge and trap methodology, high-resolution gas chromatography, and magnetic sector mass spectrometry, which gives detection limits in the low parts per trillion (ppt) range.

7. REGULATIONS AND ADVISORIES

Table 7-l summarizes international, national, and state regulations and guidelines on human exposure to carbon disulfide. No EPA, NTP, or IARC cancer classifications were reported for carbon disulfide.

ATSDR has derived an inhalation MRL of 0.3 ppm for chronic-duration inhalation exposure in humans exposed 8 hours/day, 5 days/week; this MRL is based on a LOAEL of 7.6 ppm (Johnson et al. 1983). In addition, ATSDR has derived an oral MRL of 0.01 mg/kg/day for acute-duration oral exposure; this MRL is based on a LOAEL of 3 mg/kg/day in mice (Masuda et al. 1986).

An oral reference dose (RfD) of 0.1 mg/kg/day has been derived by EPA for carbon disulfide (IRIS 1995). The RfD is based on a NOAEL of 11 mg/kg/day carbon disulfide for fetal toxicity in rabbits following inhalation exposure (Hardin et al. 1981). An inhalation reference concentration (RfC) of 0.7 mg/m^3 (0.2 ppm) was also derived for carbon disulfide (IRIS 1995). The RfC was based on a benchmark concentration (human-equivalent) of 19.7 mg/m^3 (6.3 ppm) divided by an uncertainty factor of 30 (Johnson et al. 1983).

The Clean Air Act regulates carbon disulfide for equipment leaks of volatile organic compounds in the synthetic organic chemical manufacturing industry (EPA 1995g).

OSHA has limited the 8-hour workplace exposure to carbon disulfide to 20 ppm (OSHA 1995a). This OSHA exposure value reflects the limit that was in effect prior to the issuance of the new limits (carbon disulfide was 4 ppm (PEL), 12 ppm (STEL) and 500 ppm (IDLH)) on January 19, 1989, which were vacated by the Eleventh Circuit Court of Appeals on July 7, 1992. NIOSH has recommended a TWA of 1 ppm and a STEL of 10 ppm (NIOSH 1994). The ACGIH has recommended a TLV-TWA of 10 ppm (ACGIH 1995).

TABLE 7-1. Regulations and Guidelines Applicable to Carbon Disulfide

Agency	Description	Information	References
INTERNATIONAL			
WHO	Occupational exposure limits		WHO 1981
	TWA (male human)	10 mg/m^3 (3.2 ppm)	
	TWA (females of fertile age)	3 mg/m^3 (0.96 ppm)	
	STEL (15-minute)	60 mg/m^3 (19.2 ppm)	
NATIONAL			
Regulations:			
a. Air:			
EPA	Standards of performance for new stationary sources: requirements to prevent equipment leaks of volatile organic compounds (VOCs) in the synthetic organic chemical manufacturing industry (SOCMI) in compliance with the Clean Air Act; carbon disulfide	Yes	EPA 1995a (48 FR 48335)
OSHA	Final rule limits:		OSHA 1995a (58 FR 40191)
	PEL TWA (8-hour)	20 ppm (60 mg/m^3)	
	STEL (15-minute)	12 ppm (36 mg/m^3)	
	Skin designation	Yes	
	Max peak (30-minute)	100 ppm (300 mg/m^3)	
	Ceiling	30 ppm (90 mg/m^3)	
	IDHL	500 ppm	
b. Water:			
EPA OWRS	NPDES permit application testing requirements; toxic pollutants and hazardous substances required to be identified by existing dischargers if expected to be present	Yes	EPA 1995b (48 FR 14153)
c. Food:			
EPA	Removal of carbon disulfide's exemption from the requirement of a tolerance in or on raw agricultural commodities; 40 CFR 180 amended	Yes	EPA 1995c (54 FR 6130)
	Tolerances for the nematicide, insectide, and fungicide from the application of sodium tetrathiocabonate in or on grapefruit, grapes, lemons, and oranges	0.1 ppm	EPA 1995d (58 FR 33771)

TABLE 7-1. Regulations and Guidelines Applicable to Carbon Disulfide (*continued*)

Agency	Description	Information	References
NATIONAL (Cont.)			
d. Other:			
DOT	DOT-Hazard: Flammable Liquid Label: Flammable Liquid	Yes	DOT 1995a (55 FR 52472)
	DOT-IMO: Flammable Liquid Label: Flammable Liquid, Poison	Yes	DOT 1995b (55 FR 52582)
EPA OERR	CERCLA reportable quantity	100 pounds (45.4 kg)	EPA 1995e (50 FR 13474)
	Extremely hazardous substance TPQ	10,000 pounds	EPA 1995f (55 FR 5546)
EPA OSW	Designation of hazardous substance under section 311(b)(2)(A) of the Federal Water Pollution Control Act	Yes	EPA 1995g (54 FR 33482)
	Listed as toxic waste from nonspecific sources: spent nonhalogenated solvents such as carbon disulfide; spent solvent mixtures containing a total of at least 10% (by volume) of carbon disulfide before use; and still bottoms from the recovery of above nonhalogenated solvent and solvent mixtures; this item was listed as a hazardous waste due to its toxicity and ignitability	Yes	EPA 1995h (46 FR 4618)
	Listed as hazardous waste: discarded commercial chemical products, off-specification species, container residues, and spill residues thereof	Yes	EPA 1995i (45 FR 78529)
	Listed as a hazardous constituent	Yes	EPA 1995j (56 FR 7568)
	Groundwater monitoring requirement list	Yes	EPA 1995k (52 FR 25947)
EPA OTS	Toxic chemical release reporting: community-right-to-know	Yes	EPA 1995l (60 FR 34187)
OSHA	Meets criteria for OSHA medical records rule	Yes	OSHA 1995b (48 FR 35736)
Guidelines:			
a. Air:			
ACGIH	TLV TWA	10 ppm (31 mg/m^3)	ACGIH 1995
	Skin designation	Yes	ACGIH 1995
	BEI: 2-thiothiazolidine-4-carboxylic acid in urine at the end of shift	5 mg/g creatinine	ACGIH 1995

TABLE 7-1. Regulations and Guidelines Applicable to Carbon Disulfide (*continued*)

Agency	Description	Information	References
NATIONAL (Cont.)			
NIOSH	REL TWA (10-hour)	1 ppm (3 mg/m^3)	NIOSH 1994
	Skin designation	Yes	NIOSH 1994
	STEL (15-minute)	10 ppm (30 mg/m^3)	NIOSH 1994
b. Other:			
EPA	RfD (oral)	1×10^{-1} mg/kg/day	IRIS 1995
	RfC (inhalation)	7×10^{-1} mg/m^3	IRIS 1995
STATE[a]			
Regulations and Guidelines:			
a. Air:	Acceptable ambient air concentrations		NATICH 1995
Arizona	(1-hour)	0.029 ppm (0.091 mg/m^3)	
	(24-hour)	0.008 ppm (0.024 mg/m^3)	
Connecticut	(8-hour)	0.019 ppm (0.0600 mg/m^3)	
Florida	(8-hour)	0.096 ppm (0.300 mg/m^3)	
Florida	(8-hour)	0.038 ppm (0.120 mg/m^3)	
	(24-hour)	0.009 ppm (0.0280 mg/m^3)	
	(annual)	0.00 ppm (0.00001 mg/m^3)	
Louisiana	(8-hour)	0.028 ppm (0.086 mg/m^3)	
Nevada	(8-hour)	0.228 ppm (0.7140 mg/m^3)	
New York	(1-year)	0.032 ppm (0.100 mg/m^3)	
North Carolina	(24-hour)	0.060 ppm (0.186 mg/m^3)	
North Dakota	(8-hour)	0.100 ppm (0.3100 mg/m^3)	
Oklahoma	(24-hour)	0.020 ppm (0.062 mg/m^3)	
South Carolina	(24-hour)	0.048 ppm (0.150 mg/m^3)	

TABLE 7-1. Regulations and Guidelines Applicable to Carbon Disulfide (*continued*)

Agency	Description	Information	References
STATE (Cont.)			
Texas	(30-minute)	0.010 ppm (0.030 mg/m^3)	
	(annual)	0.001 ppm (0.003 mg/m^3)	
Vermont	(24-hour)	0.228 ppm (0.714 mg/m^3)	
Virginia	(24-hour)	0.166 ppm (0.520 mg/m^3)	
Washington	(24-hour)	0.032 ppm (0.099 mg/m^3)	
Wisconson	(24-hour)	0.230 ppm (0.720 mg/m^3)	
b. Water:	Drinking water quality guidelines		HSDB 1995
Arizona		830 μg/L	
Michigan		80 μg/L	
Minnesota		700 μg/L	

[a]State regulations are not necessarily applied state-wide. For specific infomation as to the areas affected by the regulations refer to NATICH 1992.

ACGIH = American Conference of Governmental Industrial Hygienists; BEI = Biological Exposure Index; CERCLA = Comprehensive Environmental Response, Compensation, and Liability Act; CFR = Code of Federal Regulations; DOT = Department of Transportation; DOT-IMO = Department of Transportation/International Maritime Organization; EPA = Environmental Protection Agency; NIOSH = National Institute for Occupational Safety and Health; NPDES = National Pollutant Discharge Elimination System; OERR = Office of Emergency and Remedial Response; OSHA = Occupational Safety and Health Administration; OSW = Office of Solid Wastes; OTS = Office of Toxic Substances; OWRS = Office of Water Regulations and Standards; PEL = Permissible Exposure Limit; REL = Recommended Exposure Limit; RfC = Reference Concentration; RfD = Reference Dose; STEL = Short-Term Exposure Limit; TLV = Threshold Limit Value; TPQ = Threshold Planning Quantity; TWA = Time-Weighted Average; WHO = World Health Organization

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9. GLOSSARY

Acute Exposure -- Exposure to a chemical for a duration of 14 days or less, as specified in the Toxicological Profiles.

Adsorption Coefficient (K_{oc}) -- The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

Adsorption Ratio (Kd) -- The amount of a chemical adsorbed by a sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

Bioconcentration Factor (BCF) -- The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

Cancer Effect Level (CEL) -- The lowest dose of chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or tumors) between the exposed population and its appropriate control.

Carcinogen -- A chemical capable of inducing cancer.

Ceiling Value -- A concentration of a substance that should not be exceeded, even instantaneously.

Chronic Exposure -- Exposure to a chemical for 365 days or more, as specified in the Toxicological Profiles.

Developmental Toxicity -- The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Embryotoxicity and Fetotoxicity -- Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the insult occurred. The terms, as used here, include malformations and variations, altered growth, and in utero death.

EPA Health Advisory -- An estimate of acceptable drinking water levels for a chemical substance based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

Immediately Dangerous to Life or Health (IDLH) -- The maximum environmental concentration of a contaminant from which one could escape within 30 min without any escape-impairing symptoms or irreversible health effects.

Intermediate Exposure -- Exposure to a chemical for a duration of 15-364 days, as specified in the Toxicological Profiles.

Immunologic Toxicity -- The occurrence of adverse effects on the immune system that may result from exposure to environmental agents such as chemicals.

In Vitro -- Isolated from the living organism and artificially maintained, as in a test tube.

In Vivo -- Occurring within the living organism.

Lethal Concentration$_{(LO)}$ (LC_{LO}) -- The lowest concentration of a chemical in air which has been reported to have caused death in humans or animals.

Lethal Concentration$_{(50)}$ (LC_{50}) -- A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose$_{(LO)}$ (LD_{LO}) -- The lowest dose of a chemical introduced by a route other than inhalation that is expected to have caused death in humans or animals.

Lethal Dose$_{(50)}$ (LD_{50}) -- The dose of a chemical which has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time$_{(50)}$ (LT_{50}) -- A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL) -- The lowest dose of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Malformations -- Permanent structural changes that may adversely affect survival, development, or function.

Minimal Risk Level -- An estimate of daily human exposure to a dose of a chemical that is likely to be without an appreciable risk of adverse noncancerous effects over a specified duration of exposure.

Mutagen -- A substance that causes mutations. A mutation is a change in the genetic material in a body cell. Mutations can lead to birth defects, miscarriages, or cancer.

Neurotoxicity -- The occurrence of adverse effects on the nervous system following exposure to chemical.

No-Observed-Adverse-Effect Level (NOAEL) -- The dose of chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Effects may be produced at this dose, but they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{ow}) -- The equilibrium ratio of the concentrations of a chemical in *n*-octanol and water, in dilute solution.

Permissible Exposure Limit (PEL) -- An allowable exposure level in workplace air averaged over an 8-hour shift.

q_1* -- The upper-bound estimate of the low-dose slope of the dose response curve as determined by the multistage procedure. The q_1* can be used to calculate an estimate of carcinogenic potency, the incremental excess cancer risk per unit of exposure (usually μg/L for water, mg/kg/day for food, and μg/m^3 for air).

Reference Dose (RfD) -- An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure of the human population to a potential hazard that is likely to be without risk of deleterious effects during a lifetime. The RfD is operationally derived from the NOAEL (from animal and human studies) by a consistent application of uncertainty factors that reflect various types of data used to estimate RfDs and an additional modifying factor, which is based on a professional judgment of the entire database on the chemical. The RfDs are not applicable to nonthreshold effects such as cancer.

Reportable Quantity (RQ) -- The quantity of a hazardous substance that is considered reportable under CERCLA. Reportable quantities are (1) 1 pound or greater or (2) for selected substances, an amount established by regulation either under CERCLA or under Sect. 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

Reproductive Toxicity -- The occurrence of adverse effects on the reproductive system that may result from exposure to a chemical. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

Short-Term Exposure Limit (STEL) -- The maximum concentration to which workers can be exposed for up to 15 min continually. No more than four excursions are allowed per day, and there must be at least 60 min between exposure periods. The daily TLV-TWA may not be exceeded.

Target Organ Toxicity -- This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen -- A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV) -- A concentration of a substance to which most workers can be exposed without adverse effect. The TLV may be expressed as a TWA, as a STEL, or as a CL.

Time-Weighted Average (TWA) -- An allowable exposure concentration averaged over a normal 8-hour workday or 40-hour workweek.

Toxic Dose (TD_{50}) -- A calculated dose of a chemical, introduced by a route other than inhalation, which is expected to cause a specific toxic effect in 50% of a defined experimental animal population.

Uncertainty Factor (UF) -- A factor used in operationally deriving the RfD from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using LOAEL data rather than NOAEL data. Usually each of these factors is set equal to 10.

APPENDIX A

ATSDR MINIMAL RISK LEVEL

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) [42 U.S.C. 9601 et seq.], as amended by the Superfund Amendments and Reauthorization Act (SARA) [Pub. L. 99–499], requires that the Agency for Toxic Substances and Disease Registry (ATSDR) develop jointly with the U.S. Environmental Protection Agency (EPA), in order of priority, a list of hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL); prepare toxicological profiles for each substance included on the priority list of hazardous substances; and assure the initiation of a research program to fill identified data needs associated with the substances.

The toxicological profiles include an examination, summary, and interpretation of available toxicological information and epidemiologic evaluations of a hazardous substance. During the development of toxicological profiles, Minimal Risk Levels (MRLs) are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. MRLs are based on noncancer health effects only and are not based on a consideration of cancer effects. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the no-observed-adverse-effect level/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (365 days and longer) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive chemical-induced end point considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for

establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as a hundredfold below levels that have been shown to be nontoxic in laboratory animals.

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology, expert panel peer reviews, and agencywide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published levels. For additional information regarding MRLs, please contact the Division of Toxicology, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road, Mailstop E-29, Atlanta, Georgia 30333.

APPENDIX A

MINIMAL RISK LEVEL WORKSHEETS

Chemical Name: **Carbon disulfide**
CAS Number: 75-15-0
Date: July 1996
Profile Status: Second Draft
Route: [X] Inhalation [ ] Oral
Duration: [ ] Acute [ ] Intermediate [X] Chronic
Graph Key: 35
Species: Human

Minimal Risk Level: 0.3 [ ] mg/kg/day [X] ppm

Reference: Johnson, BL, Boyd J, Burg JR, et al. 1983. Effects on the peripheral nervous system of workers' exposure to carbon disulfide. Neurotoxicology 4(1):53-66.

Experimental design: (human study details or strain, number of animals per exposure/control groups, sex, dose administration details):

A cohort of male viscose rayon workers exposed to carbon disulfide (n+145) were compared to a group of non-exposed artificial fiber plant workers (n=212) located on the same premises. The mean exposure period was 12.1 ± 6.9 years (mean ± SD), and individuals were divided into three groups based on previous exposure histories, job descriptions, and current carbon disulfide levels established on the basis of 8-hour personal monitors. The median carbon disulfide level for the comparison group was 0.2 ppm (0.6 mg/cu.m, duration adjusted to 0.1 mg/cu.m; while the median carbon disulfide levels of exposed individuals was 1.4, 4.1, and 7.6 ppm (3, 13, and 24 mg/cu.m; duration adjusted to 0.7, 3.0, and 5.6 mg/cu.m). The mean exposure concentration of all groups considered together was 7.3 ± 17.2 ppm (mean ± SD) (23 mg/cu.m), ranging from 0.6 to 16 ppm (1.9 to 50 mg/cu.m). Workers were excluded on the basis of excess alcohol consumption, diabetes, or elevated blood lead levels. Surface electrodes were used to measure maximum motor conduction velocity (MCV) in the ulnar and peroneal nerves, and sensory nerve conduction velocity (SCV) in the sural nerve. Both latency, and amplitude ratios were calculated. Data were presented after they adjusted for temperature and terminal distance. In addition, participant's responses to a medical questionnaire with questions relevant to both central and peripheral nervous system symptoms were tabulated. Neurophysiological test results from the comparison group were compared to the overall exposure group, as well as to the low, medium, and high exposure groups.

Effects noted in study and corresponding doses:

Peroneal MCV decreased in a dose-dependent manner with increasing carbon disulfide exposure levels. This decrease was statistically significantly in the high concentration exposure group vs. the comparison group. When MCV was stratified according to the cumulative exposure index (ppm months), a significant association was made between this index and decreased MCV. The peroneal nerve amplitude ratio was also significantly decreased in the highest exposure group. Sural SCV was decreased in exposed vs. comparison groups; however there was no dose response relationship in the three groups. The sural sensory amplitude was significantly greater in the high exposure group than in the low exposure group, however the value for the comparison group was midway between the medium and high exposure values. Therefore, the significance of this finding is unclear. No differences in the number of self-reported symptoms related to the peripheral nervous system were

found. Study limitations included the use of only one sex (not enough women to permit valid statistical analysis were identified), failure to use a biomarker (such as carbon disulfide in blood or urine), the variability of exposure concentrations, the use of median exposure concentrations for comparison that were based on job history and air and personal monitoring, and potential concurrent exposure in the workplace to hydrogen sulfide, tin oxide, zinc oxide and sulfate, sodium hydroxide, and sulfuric acid. Hydrogen sulfide levels were determined not to exceed 1 ppm. There were brief periods of high carbon disulfide exposures due to infrequent periods of breakdown in production. An appropriate exclusion of confounding factors (diabetes, alcohol consumption, blood lead levels) was used. The decrease in MCV constitutes a LOAEL of 7.6 ppm (24 mg/cu.m) which was considered minimal because the MCVs, although reduced compared to controls, were still within a range of clinically normal values. The study authors and the MRL workgroup considered these effects indicative of minimal neurotoxicity.

Dose and endpoint used for MRL derivation:

[X] 3 for use of a minimal LOAEL
[ ] 10 for extrapolation from animals to humans
[X] 10 for human variability

Was a conversion used from ppm in food or water to a mg/body weight dose? If so explain: NA

If an inhalation study in animals, list the conversion factors used in determining human equivalent dose: N/A

MRL Calculation:

LOAEL: 7.6 ppm
MRL = LOAEL /UF = 7.6/30 = 0.25 ppm, rounded to 0.3 ppm

Agency Contact (Chemical Manager): Henry Abadin, MSPH

APPENDIX A

Chemical Name: **Carbon disulfide**
CAS Number: 75-15-0
Date: July 1996
Profile Status: Second Draft
Route: [ ] Inhalation [X] Oral
Duration: [X] Acute [ ] Intermediate [ ] Chronic
Graph Key: 2
Species: Mice

Minimal Risk Level: 0.01 [X] mg/kg/day [ ] ppm

Reference: Masuda Y, Yasoshima M, and Nakayama N. 1986. Early Selective and Reversible Suppression of Cytochrome P-450-Dependent Monooxygenase of Liver Microsomes following the Administration of Low Doses of Carbon Disulfide in Mice. Biochemical Pharmacology 35(22): 3941-3948.

Experimental design:(human study details or strain, number of animals per exposure/control groups, sex, dose administration details):

The effects of carbon disulfide on the liver microsomal drug-metabolizing enzyme system and other enzyme activities were examined one hour after the oral administration of 0, 3, 30, 300 mg/kg carbon disulfide in SPF-grade male mice of the ddY-strain. Carbon disulfide was administered in olive oil. Control animals received only the vehicle. Microsomal enzyme assays included cytochrome P-450, cytochrome b5, total heme content, NADPH-P-450 reductase, NADPH-cytochrome c reductase, NADH-ferricyanide reductase, NADH-cytochrome c reductase, P-450 dependent peroxidase activity (aniline hydroxylation and N,N-dimethyl p-phenylene diamine oxidation, 7-ethoxycoumarin and 7-ethoxyresorufin-O-deethylase, phenylthiourea S-oxidase, N,N-dimethylaniline N-oxidase, N-demethylase, UDP-glucuronyltransferase, glucose-6-phosphatase, glutathione S-transferase, heme oxygenase, liver glutathione content, and conjugated diene levels. The hepatotoxic potential of carbon disulfide was examined in mice following oral administration of 500, 1000, 1500, or 2000 mg/kg carbon disulfide. Plasma glutamic pyruvic transaminase activity and liver calcium content were measured as an indicator of potential hepatotoxicity. The time-course of changes in drug-metabolizing enzyme activities was studied in mice following the oral administration of 3 and 30 mg/kg carbon disulfide.

Effects noted in study and corresponding doses:

There was no evidence of hepatotoxicity in mice administered up to 2000 mg/kg carbon disulfide. Following the oral administration of 3 or 30 mg/kg carbon disulfide, the hepatic microsomal cytochrome P-450 content and drug metabolizing enzyme activities decreased rapidly, reaching their lowest levels at 1 hour, and then gradually returning to control levels by 24 hours. The following enzyme activities were decreased: hydroxylation of aniline, O-dealkylation of p-nitroanisole, 7-ethoxycoumarin and 7-ethoxyresorufin, N-demethylation of N,N-dimethylaniline, NADPH-cytochrome P-450 reductase activity, and P-450-associated peroxidase activity. A dose-dependent decrease in total heme content and cytochrome P-450 content was observed at 30 and 300 mg/kg carbon disulfide. There were no effects on the activities of NAPH-ferricyanide reductase, NADPH-cytochrome c reductase, flavin-containing monoxygenase, UDP-glucuronyltransferase, glucose-6-phosphatase, heme oxygenase, and glutathione S-transferase. Also, the content of cytochrome b5 was not altered. The decrease in liver microsomal drug-metabolizing enzymes constitutes a minimal LOAEL of

3 mg/kg/day. The effect was considered to be minimal since the inhibition of enzyme activities was selective and reversible.

Dose and endpoint used for MRL derivation:

3 mg/kg/day, dose-dependent decreases in the activities of hepatic microsomal drug-metabolizing enzyme.

[ ] NOAEL [X] LOAEL

Uncertainty Factors used in MRL derivation:

[X] 3 for use of a minimal LOAEL
[X] 10 for extrapolation from animals to humans
[X] 10 for human variability

Was a conversion used from ppm in food or water to a mg/body weight dose?
If so, explain: N/A

If an inhalation study in animals, list the conversion factors used in determining human equivalent dose: N/A

MRL Calculation:

LOAEL: 3 mg/kg/day

MRL = LOAEL/UF = 3/300 = 0.01 mg/kg/day

Agency Contact (Chemical Manager): Henry Abadin, MSPH

APPENDIX B

USER'S GUIDE

Chapter 1

Public Health Statement

This chapter of the profile is a health effects summary written in non-technical language. Its intended audience is the general public especially people living in the vicinity of a hazardous waste site or chemical release. If the Public Health Statement were removed from the rest of the document, it would still communicate to the lay public essential information about the chemical.

The major headings in the Public Health Statement are useful to find specific topics of concern. The topics are written in a question and answer format. The answer to each question includes a sentence that will direct the reader to chapters in the profile that will provide more information on the given topic.

Chapter 2

Tables and Figures for Levels of Significant Exposure (LSE)

Tables (2-1, 2-2, and 2-3) and figures (2-1 and 2-2) are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species, minimal risk levels (MRLs) to humans for noncancer end points, and EPA's estimated range associated with an upper-bound individual lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. Use the LSE tables and figures for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of No-Observed-Adverse-Effect Levels (NOAELs), Lowest-Observed-Adverse-Effect Levels (LOAELs), or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE Table 2-1 and Figure 2-1 are shown. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

LEGEND

See LSE Table 2-1

(1) Route of Exposure One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. When sufficient data exists, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure, i.e., inhalation, oral, and dermal (LSE Table 2-1, 2-2, and 2-3, respectively). LSE figures are limited to the inhalation (LSE Figure 2-1) and oral (LSE Figure 2-2) routes. Not all substances will have data on each route of exposure and will not therefore have all five of the tables and figures.

(2) Exposure Period Three exposure periods - acute (less than 15 days), intermediate (15–364 days), and chronic (365 days or more) are presented within each relevant route of exposure. In this example, an inhalation study of intermediate exposure duration is reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Health Effect The major categories of health effects included in LSE tables and figures are death, systemic, immunological, neurological, developmental, reproductive, and cancer. NOAELs and LOAELs can be reported in the tables and figures for all effects but cancer. Systemic effects are further defined in the "System" column of the LSE table (see key number 18).

(4) Key to Figure Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 18 has been used to derive a NOAEL and a Less Serious LOAEL (also see the 2 "18r" data points in Figure 2-1).

(5) Species The test species, whether animal or human, are identified in this column. Section 2.5, "Relevance to Public Health," covers the relevance of animal data to human toxicity and Section 2.3, "Toxicokinetics," contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(6) Exposure Frequency/Duration The duration of the study and the weekly and daily exposure regimen are provided in this column. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 18), rats were exposed to 1,1,2,2-tetrachloroethane via inhalation for 6 hours per day, 5 days per week, for 3 weeks. For a more complete review of the dosing regimen refer to the appropriate sections of the text or the original reference paper, i.e., Nitschke et al. 1981.

(7) System This column further defines the systemic effects. These systems include: respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, and dermal/ocular. "Other" refers to any systemic effect (e.g., a decrease in body weight) not covered in these systems. In the example of key number 18, 1 systemic effect (respiratory) was investigated.

(8) NOAEL A No-Observed-Adverse-Effect Level (NOAEL) is the highest exposure level at which no harmful effects were seen in the organ system studied. Key number 18 reports a NOAEL of 3 ppm for the respiratory system which was used to derive an intermediate exposure, inhalation MRL of 0.005 ppm (see footnote "b").

(9) LOAEL A Lowest-Observed-Adverse-Effect Level (LOAEL) is the lowest dose used in the study that caused a harmful health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific endpoint used to quantify the adverse effect accompanies the LOAEL. The respiratory effect reported in key number 18 (hyperplasia) is a Less serious LOAEL of 10 ppm. MRLs are not derived from Serious LOAELs.

(10) Reference The complete reference citation is given in chapter 8 of the profile.

(11) CEL A Cancer Effect Level (CEL) is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases.

(12) Footnotes Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. Footnote "b" indicates the NOAEL of 3 ppm in key number 18 was used to derive an MRL of 0.005 ppm.

LEGEND

See Figure 2-1

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(13) Exposure Period The same exposure periods appear as in the LSE table. In this example, health effects observed within the intermediate and chronic exposure periods are illustrated.

(14) Health Effect These are the categories of health effects for which reliable quantitative data exists. The same health effects appear in the LSE table.

(15) Levels of Exposure concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(16) NOAEL In this example, 18r NOAEL is the critical endpoint for which an intermediate inhalation exposure MRL is based. As you can see from the LSE figure key, the open-circle symbol indicates to a NOAEL for the test species-rat. The key number 18 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 3 ppm (see entry 18 in the Table) to the MRL of 0.005 ppm (see footnote "b" in the LSE table).

(17) CEL Key number 38r is 1 of 3 studies for which Cancer Effect Levels were derived. The diamond symbol refers to a Cancer Effect Level for the test species-mouse. The number 38 corresponds to the entry in the LSE table.

(18) Estimated Upper-Bound Human Cancer Risk Levels This is the range associated with the upper-bound for lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. These risk levels are derived from the EPA's Human Health Assessment Group's upper-bound estimates of the slope of the cancer dose response curve at low dose levels (q_1*).

(19) Key to LSE Figure The Key explains the abbreviations and symbols used in the figure.

SAMPLE

1 → **TABLE 2-1. Levels of Significant Exposure to [Chemical x] – Inhalation**

Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
2 → INTERMEDIATE EXPOSURE							
3 → Systemic	5 ↓	6 ↓	7 ↓	8 ↓	9 ↓		10 ↓
4 → 18	Rat	13 wk 5d/wk 6hr/d	Resp	3[b]	10 (hyperplasia)		Nitschke et al. 1981
CHRONIC EXPOSURE							
Cancer						11 ↓	
38	Rat	18 mo 5d/wk 7hr/d				20 (CEL, multiple organs)	Wong et al. 1982
39	Rat	89–104 wk 5d/wk 6hr/d				10 (CEL, lung tumors, nasal tumors)	NTP 1982
40	Mouse	79–103 wk 5d/wk 6hr/d				10 (CEL, lung tumors, hemangiosarcomas)	NTP 1982

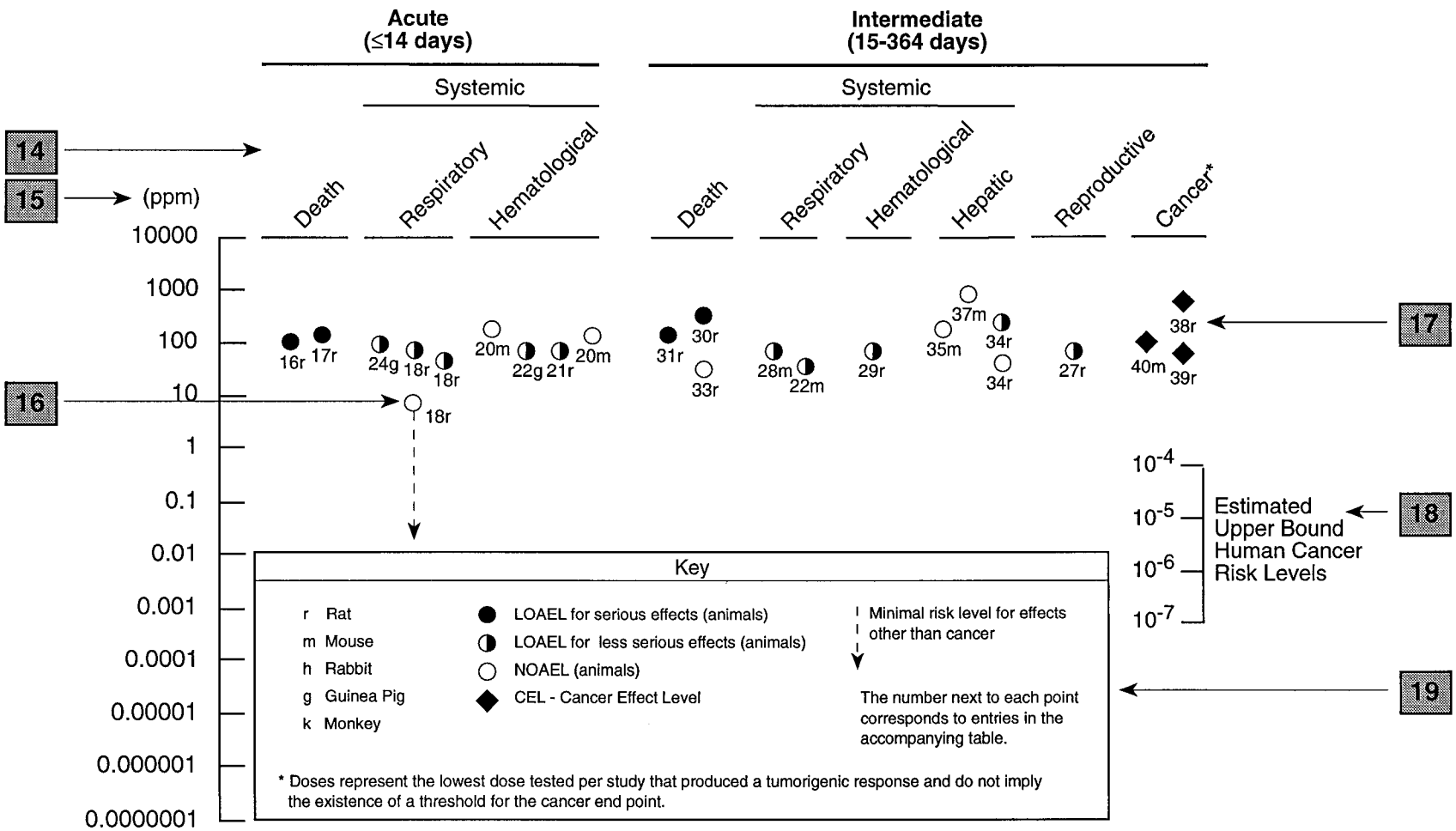
[a] The number corresponds to entries in Figure 2-1.

12 → [b] Used to derive an intermediate inhalation Minimal Risk Level (MRL) of 5 x 10^{-3} ppm; dose adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animal to humans, 10 for human variability).

CEL = cancer effect level; d = days(s); hr = hour(s); LOAEL = lowest-observed-adverse-effect level; mo = month(s); NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s)

SAMPLE

13 → **Figure 2-1. Levels of Significant Exposure to [Chemical X] – Inhalation**



Chapter 2 (Section 2.5)

Relevance to Public Health

The Relevance to Public Health section provides a health effects summary based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information. This summary is designed to present interpretive, weight-of-evidence discussions for human health end points by addressing the following questions.

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

The section covers end points in the same order they appear within the Discussion of Health Effects by Route of Exposure section, by route (inhalation, oral, dermal) and within route by effect. Human data are presented first, then animal data. Both are organized by duration (acute, intermediate, chronic). *In vitro* data and data from parenteral routes (intramuscular, intravenous, subcutaneous, etc.) are also considered in this section. If data are located in the scientific literature, a table of genotoxicity information is included.

The carcinogenic potential of the profiled substance is qualitatively evaluated, when appropriate, using existing toxicokinetic, genotoxic, and carcinogenic data. ATSDR does not currently assess cancer potency or perform cancer risk assessments. Minimal risk levels (MRLs) for noncancer end points (if derived) and the end points from which they were derived are indicated and discussed.

Limitations to existing scientific literature that prevent a satisfactory evaluation of the relevance to public health are identified in the Data Needs section.

Interpretation of Minimal Risk Levels

Where sufficient toxicologic information is available, we have derived minimal risk levels (MRLs) for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action; but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans. They should help physicians and public health officials determine the safety of a community living near a chemical emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Chapter 2.5, "Relevance to Public Health," contains basic information known about the substance. Other sections such as 2.7, "Interactions with Other Substances," and 2.8, "Populations that are Unusually Susceptible" provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses for lifetime exposure (RfDs).

To derive an MRL, ATSDR generally selects the most sensitive endpoint which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen endpoint are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest NOAEL that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor (UF) of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a substance-specific MRL are provided in the footnotes of the LSE Tables.

APPENDIX C

ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
ADME	Absorption, Distribution, Metabolism, and Excretion
AML	acute myeloid leukemia
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
BCF	bioconcentration factor
BEI	Biological Exposure Index
BSC	Board of Scientific Counselors
C	Centigrade
CDC	Centers for Disease Control
CEL	Cancer Effect Level
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
Ci	curie
CLP	Contract Laboratory Program
cm	centimeter
CML	chronic myeloid leukemia
CNS	central nervous system
d	day
DHEW	Department of Health, Education, and Welfare
DHHS	Department of Health and Human Services
DOL	Department of Labor
ECG	electrocardiogram
EEG	electroencephalogram
EPA	Environmental Protection Agency
EKG	see ECG
F	Fahrenheit
F_1	first filial generation
FAO	Food and Agricultural Organization of the United Nations
FEMA	Federal Emergency Management Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
fpm	feet per minute
ft	foot
FR	*Federal Register*
g	gram
GC	gas chromatography
gen	generation
HPLC	high-performance liquid chromatography
hr	hour
IDLH	Immediately Dangerous to Life and Health
IARC	International Agency for Research on Cancer
ILO	International Labor Organization
in	inch
Kd	adsorption ratio

kg	kilogram
kkg	metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{Lo}	lethal concentration, low
LC_{50}	lethal concentration, 50% kill
LD_{Lo}	lethal dose, low
LD_{50}	lethal dose, 50% kill
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
m	meter
MA	trans,trans-muconic acid
mCi	millicurie
mg	milligram
min	minute
mL	milliliter
mm	millimeter
mm Hg	millimeters of mercury
mmol	millimole
mo	month
mppcf	millions of particles per cubic foot
MRL	Minimal Risk Level
MS	mass spectrometry
NCE	normochromatic erythrocytes
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NIOSHTIC	NIOSH's Computerized Information Retrieval System
ng	nanogram
nm	nanometer
NHANES	National Health and Nutrition Examination Survey
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NOES	National Occupational Exposure Survey
NOHS	National Occupational Hazard Survey
NPL	National Priorities List
NRC	National Research Council
NTIS	National Technical Information Service
NTP	National Toxicology Program
OSHA	Occupational Safety and Health Administration
PEL	permissible exposure limit
PCE	polychromatic erythrocytes
pg	picogram
pmol	picomole
PHS	Public Health Service
PMR	proportionate mortality ratio
ppb	parts per billion
ppm	parts per million

ppt	parts per trillion
REL	recommended exposure limit
RfD	Reference Dose
RTECS	Registry of Toxic Effects of Chemical Substances
sec	second
SCE	sister chromatid exchange
SIC	Standard Industrial Classification
SMR	standard mortality ratio
STEL	short term exposure limit
STORET	STORAGE and RETRIEVAL
TLV	threshold limit value
TSCA	Toxic Substances Control Act
TRI	Toxics Release Inventory
TWA	time-weighted average
UMDNJ	University of Medicine and Dentistry New Jersey
U.S.	United States
UF	uncertainty factor
yr	year
WHO	World Health Organization
wk	week
$>$	greater than
$\geq$	greater than or equal to
$=$	equal to
$<$	less than
$\leq$	less than or equal to
%	percent
α	alpha
β	beta
δ	delta
γ	gamma
μm	micrometer
μg	microgram

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TOXICOLOGICAL PROFILE FOR CARBON TETRACHLORIDE

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

August 2005

DISCLAIMER

The use of company or product name(s) is for identification only and does not imply endorsement by the Agency for Toxic Substances and Disease Registry.

UPDATE STATEMENT

A Toxicological Profile for Carbon Tetrachloride, Draft for Public Comment was released in September 2003. This edition supersedes any previously released draft or final profile.

Toxicological profiles are revised and republished as necessary. For information regarding the update status of previously released profiles, contact ATSDR at:

Agency for Toxic Substances and Disease Registry
Division of Toxicology/Toxicology Information Branch
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FOREWORD

This toxicological profile is prepared in accordance with guidelines* developed by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA). The original guidelines were published in the *Federal Register* on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for the hazardous substance described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a hazardous substance's toxicologic properties. Other pertinent literature is also presented, but is described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

The focus of the profiles is on health and toxicologic information; therefore, each toxicological profile begins with a public health statement that describes, in nontechnical language, a substance's relevant toxicological properties. Following the public health statement is information concerning levels of significant human exposure and, where known, significant health effects. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to protection of public health are identified by ATSDR and EPA.

Each profile includes the following:

(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a hazardous substance to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects;

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure that present a significant risk to human health of acute, subacute, and chronic health effects; and

(C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

The principal audiences for the toxicological profiles are health professionals at the Federal, State, and local levels; interested private sector organizations and groups; and members of the public.

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed. Staff of the Centers for Disease Control and Prevention and other Federal scientists have also reviewed the profile. In addition, this profile has been peer-reviewed by a nongovernmental panel and was made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

Julie Louise Gerberding, M.D., M.P.H.
Administrator
Agency for Toxic Substances and
Disease Registry

*Legislative Background

The toxicological profiles are developed in response to the Superfund Amendments and Reauthorization Act (SARA) of 1986 (Public Law 99-499) which amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). This public law directed ATSDR to prepare toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. The availability of the revised priority list of 275 hazardous substances was announced in the *Federal Register* on November 7, 2003 (68 FR 63098) . For prior versions of the list of substances, see *Federal Register* notices dated April 17, 1987 (52 FR 12866); October 20, 1988 (53 FR 41280); October 26, 1989 (54 FR 43619); October 17,1990 (55 FR 42067); October 17, 1991 (56 FR 52166); October 28, 1992 (57 FR 48801); February 28, 1994 (59 FR 9486); April 29, 1996 (61 FR 18744); November 17, 1997 (62 FR 61332); October 21, 1999(64 FR 56792) and October 25, 2001 (66 FR 54014). Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list.

QUICK REFERENCE FOR HEALTH CARE PROVIDERS

Toxicological Profiles are a unique compilation of toxicological information on a given hazardous substance. Each profile reflects a comprehensive and extensive evaluation, summary, and interpretation of available toxicologic and epidemiologic information on a substance. Health care providers treating patients potentially exposed to hazardous substances will find the following information helpful for fast answers to often-asked questions.

Primary Chapters/Sections of Interest

Chapter 1: Public Health Statement: The Public Health Statement can be a useful tool for educating patients about possible exposure to a hazardous substance. It explains a substance's relevant toxicologic properties in a nontechnical, question-and-answer format, and it includes a review of the general health effects observed following exposure.

Chapter 2: Relevance to Public Health: The Relevance to Public Health Section evaluates, interprets, and assesses the significance of toxicity data to human health.

Chapter 3: Health Effects: Specific health effects of a given hazardous compound are reported by type of health effect (death, systemic, immunologic, reproductive), by route of exposure, and by length of exposure (acute, intermediate, and chronic). In addition, both human and animal studies are reported in this section.
NOTE: Not all health effects reported in this section are necessarily observed in the clinical setting. Please refer to the Public Health Statement to identify general health effects observed following exposure.

Pediatrics: Four new sections have been added to each Toxicological Profile to address child health issues:

Section 1.6 How Can (Chemical X) Affect Children?
Section 1.7 How Can Families Reduce the Risk of Exposure to (Chemical X)?
Section 3.7 Children's Susceptibility
Section 6.6 Exposures of Children

Other Sections of Interest:

Section 3.8 Biomarkers of Exposure and Effect
Section 3.11 Methods for Reducing Toxic Effects

ATSDR Information Center

Phone: 1-888-42-ATSDR or (404) 498-0110 ***Fax:*** (770) 488-4178
E-mail: atsdric@cdc.gov ***Internet***: http://www.atsdr.cdc.gov

The following additional material can be ordered through the ATSDR Information Center:

Case Studies in Environmental Medicine: Taking an Exposure History—The importance of taking an exposure history and how to conduct one are described, and an example of a thorough exposure history is provided. Other case studies of interest include *Reproductive and Developmental*

Hazards; Skin Lesions and Environmental Exposures; Cholinesterase-Inhibiting Pesticide Toxicity; and numerous chemical-specific case studies.

Managing Hazardous Materials Incidents is a three-volume set of recommendations for on-scene (prehospital) and hospital medical management of patients exposed during a hazardous materials incident. Volumes I and II are planning guides to assist first responders and hospital emergency department personnel in planning for incidents that involve hazardous materials. Volume III—*Medical Management Guidelines for Acute Chemical Exposures*—is a guide for health care professionals treating patients exposed to hazardous materials.

Fact Sheets (ToxFAQs) provide answers to frequently asked questions about toxic substances.

Other Agencies and Organizations

The National Center for Environmental Health (NCEH) focuses on preventing or controlling disease, injury, and disability related to the interactions between people and their environment outside the workplace. Contact: NCEH, Mailstop F-29, 4770 Buford Highway, NE, Atlanta, GA 30341-3724 • Phone: 770-488-7000 • FAX: 770-488-7015.

The National Institute for Occupational Safety and Health (NIOSH) conducts research on occupational diseases and injuries, responds to requests for assistance by investigating problems of health and safety in the workplace, recommends standards to the Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA), and trains professionals in occupational safety and health. Contact: NIOSH, 200 Independence Avenue, SW, Washington, DC 20201 • Phone: 800-356-4674 or NIOSH Technical Information Branch, Robert A. Taft Laboratory, Mailstop C-19, 4676 Columbia Parkway, Cincinnati, OH 45226-1998 • Phone: 800-35-NIOSH.

The National Institute of Environmental Health Sciences (NIEHS) is the principal federal agency for biomedical research on the effects of chemical, physical, and biologic environmental agents on human health and well-being. Contact: NIEHS, PO Box 12233, 104 T.W. Alexander Drive, Research Triangle Park, NC 27709 • Phone: 919-541-3212.

Referrals

The Association of Occupational and Environmental Clinics (AOEC) has developed a network of clinics in the United States to provide expertise in occupational and environmental issues. Contact: AOEC, 1010 Vermont Avenue, NW, #513, Washington, DC 20005 • Phone: 202-347-4976 • FAX: 202-347-4950 • e-mail: AOEC@AOEC.ORG • Web Page: http://www.aoec.org/.

The American College of Occupational and Environmental Medicine (ACOEM) is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. Contact: ACOEM, 25 Northwest Point Boulevard, Suite 700, Elk Grove Village, IL 60007-1030 • Phone: 847-818-1800 • FAX: 847-818-9266.

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THE PROFILE HAS UNDERGONE THE FOLLOWING ATSDR INTERNAL REVIEWS:

1. Health Effects Review. The Health Effects Review Committee examines the health effects chapter of each profile for consistency and accuracy in interpreting health effects and classifying end points.

2. Minimal Risk Level Review. The Minimal Risk Level Workgroup considers issues relevant to substance-specific Minimal Risk Levels (MRLs), reviews the health effects database of each profile, and makes recommendations for derivation of MRLs.

3. Data Needs Review. The Research Implementation Branch reviews data needs sections to assure consistency across profiles and adherence to instructions in the Guidance.

4. Green Border Review. Green Border review assures the consistency with ATSDR policy.

PEER REVIEW

A peer review panel was assembled for CARBON TETRACHLORIDE. The panel consisted of the following members:

1. Finis Cavender, Ph.D., DABT, Consultant in Toxicology, CEI, Greer, South Carolina;

2. Lisa Kamendulis, Ph.D., Assistant Scientist, Department of Pharmacology and Toxicology, Indiana University School of Medicine; Associate Director, Indiana State Department of Toxicology, Indianapolis, Indiana; and

3. Julie Stickney, Ph.D., Principal Scientist, ARCADIS G&M, Inc., Portland, Maine.

These experts collectively have knowledge of carbon tetrachloride's physical and chemical properties, toxicokinetics, key health end points, mechanisms of action, human and animal exposure, and quantification of risk to humans. All reviewers were selected in conformity with the conditions for peer review specified in Section 104(I)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

Scientists from the Agency for Toxic Substances and Disease Registry (ATSDR) have reviewed the peer reviewers' comments and determined which comments will be included in the profile. A listing of the peer reviewers' comments not incorporated in the profile, with a brief explanation of the rationale for their exclusion, exists as part of the administrative record for this compound.

The citation of the peer review panel should not be understood to imply its approval of the profile's final content. The responsibility for the content of this profile lies with the ATSDR.

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1. PUBLIC HEALTH STATEMENT

This public health statement tells you about carbon tetrachloride and the effects of exposure to it.

The Environmental Protection Agency (EPA) identifies the most serious hazardous waste sites in the nation. These sites are then placed on the National Priorities List (NPL) and are targeted for long-term federal clean-up activities. Carbon tetrachloride has been found in at least 430 of the 1,662 current or former NPL sites. Although the total number of NPL sites evaluated for this substance is not known, the possibility exists that the number of sites at which carbon tetrachloride is found may increase in the future as more sites are evaluated. This information is important because these sites may be sources of exposure and exposure to this substance may harm you.

When a substance is released either from a large area, such as an industrial plant, or from a container, such as a drum or bottle, it enters the environment. Such a release does not always lead to exposure. You can be exposed to a substance only when you come in contact with it. You may be exposed by breathing, eating, or drinking the substance, or by skin contact.

If you are exposed to carbon tetrachloride, many factors will determine whether you will be harmed. These factors include the dose (how much), the duration (how long), and how you come in contact with it. You must also consider any other chemicals you are exposed to and your age, sex, diet, family traits, lifestyle, and state of health.

1.1 WHAT IS CARBON TETRACHLORIDE?

Carbon tetrachloride is a clear liquid that evaporates very easily. Most carbon tetrachloride that escapes to the environment is therefore found as a gas. Carbon tetrachloride does not easily burn. Carbon tetrachloride has a sweet odor, and most people can begin to smell it in air when the concentration reaches 10 parts carbon tetrachloride per million parts of air (ppm). It is not

known whether people can taste it or, if they can, at what level. Carbon tetrachloride is a manufactured chemical and does not occur naturally in the environment,

Carbon tetrachloride has been produced in large quantities to make refrigeration fluid and propellants for aerosol cans. Since many refrigerants and aerosol propellants have been found to affect the earth's ozone layer, the production of these chemicals is being phased out. Consequently, the manufacture and use of carbon tetrachloride has declined a great deal.

In the past, carbon tetrachloride was widely used as a cleaning fluid (in industry and dry cleaning establishments as a degreasing agent, and in households as a spot remover for clothing, furniture, and carpeting). Carbon tetrachloride was also used in fire extinguishers and as a fumigant to kill insects in grain. Most of these uses were discontinued in the mid-1960s. Until recently, carbon tetrachloride was used as a pesticide, but this was stopped in 1986.

Further information on the properties and uses of carbon tetrachloride can be found in Chapters 4, 5, and 6.

1.2 WHAT HAPPENS TO CARBON TETRACHLORIDE WHEN IT ENTERS THE ENVIRONMENT?

Because carbon tetrachloride evaporates easily, most of the compound released to the environment during its production and use reaches the air, where it is found mainly as a gas. It can remain in air for several years before it is broken down to other chemicals. Small amounts of carbon tetrachloride are found in surface water. Because it evaporates easily, much of it will move from surface water to the air within a few days or weeks. However, it may be trapped in groundwater for longer periods. Carbon tetrachloride is not expected to stick to soil particles. If spilled onto the ground, much of it will evaporate to the air. Some of it may also go into groundwater, where it can remain for months before it is broken down to other chemicals. It is not expected to build up in fish. We do not know if it builds up in plants.

Further information on what happens to carbon tetrachloride in the environment may be found in Chapters 5 and 6.

1.3 HOW MIGHT I BE EXPOSED TO CARBON TETRACHLORIDE?

Very low background levels of carbon tetrachloride are found in air, water, and soil because of past and present releases. Concentrations in air of 0.1 part carbon tetrachloride per billion parts of air (ppb) are common around the world, with somewhat higher levels often found (0.2–0.6 ppb) in cities. Carbon tetrachloride is also found in some drinking water supplies, usually at concentrations less than 0.5 ppb. Exposure to levels of carbon tetrachloride higher than these typical "background" levels is likely to occur only at specific industrial locations where carbon tetrachloride is still used or near chemical waste sites where emissions into air, water, or soil are not properly controlled. Exposure at such sites could occur by breathing carbon tetrachloride present in the air, by drinking water contaminated with carbon tetrachloride, or by getting soil contaminated with carbon tetrachloride on the skin. Young children may also be exposed if they eat soil that contains carbon tetrachloride. Carbon tetrachloride has been found in water or soil at about 26% of the waste sites investigated under Superfund, at concentrations ranging from less than 50 to over 1,000 ppb.

People who work with carbon tetrachloride are likely to receive the greatest exposure to the compound. The National Institute for Occupational Safety and Health (NIOSH) estimates that 58,208 workers are potentially exposed to carbon tetrachloride in the United States. The average daily intake of carbon tetrachloride for the general population is estimated to be 0.1 microgram (µg per kg of body weight). The estimated average daily amount that the general population may drink in water is 0.01 µg per kg of body weight.

Further information on the ways that humans can be exposed to carbon tetrachloride is presented in Chapter 6.

1.4 HOW CAN CARBON TETRACHLORIDE ENTER AND LEAVE MY BODY?

Carbon tetrachloride can enter your body through your lungs if you breathe air containing carbon tetrachloride, or through your stomach and intestines if you swallow food or water containing carbon tetrachloride. Carbon tetrachloride can also pass through the skin into the body. When you inhale carbon tetrachloride, over 30–40% of what you inhale enters your body, where most of it temporarily accumulates in body fat. Some can enter the kidney, liver, brain, lungs, and skeletal muscle. When you drink water contaminated with carbon tetrachloride, about 85–91% of it can enter your body. Much of the compound that enters your body when you breathe it or drink water contaminated with it leaves your body quickly, and a lot of it can be found in your breath within a few hours. Animal studies have shown that under differing conditions, 34–75% of carbon tetrachloride leaves the body in expired air, 20–62% leaves the body in feces, and only low amounts leave the body in the urine. Animal studies also suggest that it may take weeks for the remainder of the compound in the body to be eliminated, especially that which has entered the body fat. Most of the carbon tetrachloride is eliminated from your body unchanged, but some of it may be changed to other chemicals before removal from the body (for example, chloroform, hexachloroethane, and carbon dioxide). Chloroform and hexachloroethane may themselves cause harmful effects.

Further information on how carbon tetrachloride enters and leaves the body is presented in Chapter 3.

1.5 HOW CAN CARBON TETRACHLORIDE AFFECT MY HEALTH?

Scientists use many tests to protect the public from harmful effects of toxic chemicals and to find ways for treating persons who have been harmed.

One way to learn whether a chemical will harm people is to determine how the body absorbs, uses, and releases the chemical. For some chemicals, animal testing may be necessary. Animal testing may also help identify health effects such as cancer or birth defects. Without laboratory animals, scientists would lose a basic method for getting information needed to make wise

decisions that protect public health. Scientists have the responsibility to treat research animals with care and compassion. Scientists must comply with strict animal care guidelines because laws today protect the welfare of research animals.

Most information on the health effects of carbon tetrachloride in humans comes from cases where people have been exposed to relatively high levels of carbon tetrachloride, either only once or for a short period, for example, by accidental poisoning or by working with the chemical in a confined space without ventilation. Experiments have not been performed on the effects of long-term exposure of humans to low levels of carbon tetrachloride, so the human health effects of such exposures are not known.

The liver is especially sensitive to carbon tetrachloride since it contains a large amount of the enzymes that change the form of the chemical. Some of the breakdown products may attack cell proteins, interfering with the functions of the liver cells. Products that attack cell membranes may result in the death of the cells. In mild cases, the liver becomes swollen and tender, and fat builds up inside the organ. In severe cases, liver cells may be damaged or destroyed, leading to a decrease in liver function. Such effects are usually reversible if exposure is not too high or too long.

The kidney is also sensitive to carbon tetrachloride. Less urine may be formed, leading to a buildup of water in the body (especially in the lungs) and buildup of waste products in the blood. Kidney failure often was the main cause of death in people who died after very high exposure to carbon tetrachloride. Long-term breathing exposure to carbon tetrachloride worsened age-related kidney disease in rats.

Fortunately, if injuries to the liver and kidney are not too severe, these effects eventually disappear after exposure stops. This is because both organs can repair damaged cells and replace dead cells. Function usually returns to normal within a few days or a few weeks after the exposure has stopped.

After exposure to high levels of carbon tetrachloride, the nervous system, including the brain, is affected. Such exposure can be fatal. The immediate effects are usually signs of intoxication, including headache, dizziness, and sleepiness perhaps accompanied by nausea and vomiting. These effects usually disappear within 1–2 days after exposure stops. In severe cases, stupor or even coma can result, and permanent damage to nerve cells can occur.

Carbon tetrachloride also causes effects on other tissues of the body, but these are not usually as common or important as the effects on the liver, kidney, and brain.

There have been no studies of the effects of carbon tetrachloride on reproduction in humans, but studies in rats showed that long-term inhalation may cause decreased fertility.

Studies in animals have shown that swallowing or breathing carbon tetrachloride over a period of years increases the frequency of liver tumors. Mice breathing carbon tetrachloride also developed tumors of the adrenal gland. Studies have not been performed to determine whether swallowing or breathing carbon tetrachloride causes tumors in humans, but it should be assumed that carbon tetrachloride could produce cancer. The Department of Health and Human Services (DHHS) has determined that carbon tetrachloride may reasonably be anticipated to be a carcinogen (i.e., cause cancer). The International Agency for Research on Cancer (IARC) has classified carbon tetrachloride in Group 2B, possibly carcinogenic to humans. EPA has determined that carbon tetrachloride is a probable human carcinogen.

Many reported cases of carbon tetrachloride toxicity are associated with drinking alcohol. The frequent drinking of alcoholic beverages increases the danger of organ damage from carbon tetrachloride exposure. This enhanced effect has been shown in situations in which a group of workers were exposed to carbon tetrachloride in air, but only those who were heavy consumers of alcohol became ill.

Further information on the health effects of carbon tetrachloride may be found in Chapter 3.

1.6 HOW CAN CARBON TETRACHLORIDE AFFECT CHILDREN?

This section discusses potential health effects in humans from exposures during the period from conception to maturity at 18 years of age.

Children and adults may be exposed to low levels of carbon tetrachloride in drinking water. Small children who live near factories that produce or use carbon tetrachloride could accidentally eat some of the chemical by putting dirty hands in their mouths, but the amount of carbon tetrachloride in the soil is thought to be too low to be harmful. Carbon tetrachloride is no longer used in consumer products, but children could breathe in vapors if households are still using old supplies.

It is not known if the way in which carbon tetrachloride is absorbed into and eliminated from the body is different in children than it is in adults, but the processes are likely to be similar. Compared to adults, young children have lower amounts of the enzyme that converts carbon tetrachloride to a harmful chemical. The health effects of carbon tetrachloride have not been studied in children, but they are likely to be similar to those seen in adults exposed to the chemical.

There is no direct evidence that maternal exposure to carbon tetrachloride has a harmful effect on the fetus in humans. A few human survey-type studies suggest that maternal drinking water exposure to carbon tetrachloride might possibly be related to certain birth defects, such as low birthweight and small size at birth. Information from animal studies indicates that carbon tetrachloride may cause early fetal deaths, but does not cause birth defects in babies surviving to term. However, these animal studies did not test for neurological damage in exposed newborn babies.

One study calculated that carbon tetrachloride is likely to pass from the maternal circulation into breast milk. Thus, it is possible that children could be exposed to carbon tetrachloride from breast feeding, but the levels of exposure are likely to be low.

Further information on the health effects of carbon tetrachloride in children may be found in Chapter 3.

1.7 HOW CAN FAMILIES REDUCE THE RISK OF EXPOSURE TO CARBON TETRACHLORIDE?

If your doctor finds that you have been exposed to substantial amounts of carbon tetrachloride, ask whether your children might also have been exposed. Your doctor might need to ask your state health department to investigate.

Although most consumer uses of carbon tetrachloride have been banned, children may be exposed to carbon tetrachloride in old consumer household cleaning products. Removing these old containers will reduce your family's risk of exposure to carbon tetrachloride. Household chemicals should be stored out of the reach of children to prevent accidental poisonings and skin burns. Always store household chemicals in their original containers. Never store household chemicals in containers that children would find attractive to eat and drink from, such as old soda bottles. Keep your poison control center's number next to your phone.

Sometimes older children sniff household chemicals in an attempt to get high. Your children may be exposed to carbon tetrachloride by intentionally inhaling products containing it. Talk with your children about the dangers of sniffing chemicals.

Further information on reducing the risk of exposure to carbon tetrachloride can be found in Chapter 3.

1.8 IS THERE A MEDICAL TEST TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO CARBON TETRACHLORIDE?

Several very sensitive and specific tests can detect carbon tetrachloride in exposed persons. The most convenient way is simply to measure carbon tetrachloride in exhaled air, but carbon tetrachloride can also be measured in blood, fat, or other tissues. Because special equipment is

needed, these tests are not routinely performed in doctors' offices, but your doctor can refer you to where you can obtain such a test. Although these tests can show that a person has been exposed to carbon tetrachloride, the test results cannot be used to reliably predict whether any bad health effects might result. Because carbon tetrachloride leaves the body fairly quickly, these methods are best suited to detecting exposures that have occurred within the last several days.

Further information on how carbon tetrachloride can be measured in exposed humans is given in Chapter 7.

1.9 WHAT RECOMMENDATIONS HAS THE FEDERAL GOVERNMENT MADE TO PROTECT HUMAN HEALTH?

The federal government develops regulations and recommendations to protect public health. Regulations *can* be enforced by law. The EPA, the Occupational Safety and Health Administration (OSHA), and the Food and Drug Administration (FDA) are some federal agencies that develop regulations for toxic substances. Recommendations provide valuable guidelines to protect public health, but *cannot* be enforced by law. The Agency for Toxic Substances and Disease Registry (ATSDR) and the National Institute for Occupational Safety and Health (NIOSH) are two federal organizations that develop recommendations for toxic substances.

Regulations and recommendations can be expressed as “not-to-exceed” levels, that is, levels of a toxic substance in air, water, soil, or food that do not exceed a critical value that is usually based on levels that affect animals; they are then adjusted to levels that will help protect humans. Sometimes these not-to-exceed levels differ among federal organizations because they used different exposure times (an 8-hour workday or a 24-hour day), different animal studies, or other factors.

Recommendations and regulations are also updated periodically as more information becomes available. For the most current information, check with the federal agency or organization that

provides it. Some regulations and recommendations for carbon tetrachloride include the following:

To protect the general public from exposure to carbon tetrachloride, the federal government has limited or banned the use of this compound in most common household products and fire extinguishers, and has discontinued its use as a pesticide. To protect workers who use carbon tetrachloride while on the job, the OSHA has set a maximum concentration limit in workplace air of 10 ppm for an 8-hour workday over a 40-hour work week. EPA has also set limits on how much carbon tetrachloride can be released from an industrial plant into waste water and is preparing to set limits on how much carbon tetrachloride can escape from an industrial plant into outside air. To ensure that drinking water supplies are safe, EPA has set a Maximum Contaminant Level (MCL) for carbon tetrachloride of 5 parts per billion (ppb), based on analytical detection limits in drinking water. Because carbon tetrachloride is possibly carcinogenic to humans, a Maximum Contaminant Level Goal (MCLG) of zero has been proposed. More detailed information on federal and state regulations regarding carbon tetrachloride may be found in Chapter 8.

1.10 WHERE CAN I GET MORE INFORMATION?

If you have any more questions or concerns, please contact your community or state health or environmental quality department, or contact ATSDR at the address and phone number below.

ATSDR can also tell you the location of occupational and environmental health clinics. These clinics specialize in recognizing, evaluating, and treating illnesses that result from exposure to hazardous substances.

Toxicological profiles are also available on-line at www.atsdr.cdc.gov and on CD-ROM. You may request a copy of the ATSDR ToxProfiles™ CD-ROM by calling the toll-free information

and technical assistance number at 1-888-42ATSDR (1-888-422-8737), by e-mail at atsdric@cdc.gov, or by writing to:

Agency for Toxic Substances and Disease Registry
Division of Toxicology
1600 Clifton Road NE
Mailstop F-32
Atlanta, GA 30333
Fax: 1-770-488-4178

Organizations for-profit may request copies of final Toxicological Profiles from the following:

National Technical Information Service (NTIS)
5285 Port Royal Road
Springfield, VA 22161
Phone: 1-800-553-6847 or 1-703-605-6000
Web site: http://www.ntis.gov/

2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO CARBON TETRACHLORIDE IN THE UNITED STATES

Carbon tetrachloride is a solvent that has been used in the past as a cleaning fluid or degreasing agent, as a grain fumigant, and industrially in the synthesis of refrigeration fluid and propellants for aerosol cans. Although most of these uses have been discontinued, the possibility still exists for carbon tetrachloride to be released to the environment, primarily through industrial processes or old bottles of cleaning agents containing carbon tetrachloride that may still be in the home. Degradation of carbon tetrachloride occurs slowly in the environment, which contributes to the accumulation of the chemical in the atmosphere as well as the groundwater. Carbon tetrachloride is widely dispersed and persistent in the environment, but is not detected frequently in foods.

The general population is not likely to be exposed to large amounts of carbon tetrachloride. Populations living within or very near waste sites, or areas of heavy carbon tetrachloride use would have an increased risk of exposure from contaminated media (air, water, or soil). Those likely to receive the highest levels of exposure are those who are involved in the production, formulation, handling, and application of carbon tetrachloride. Inhalation appears to be the major route of exposure for workers and also for the general population, which may be exposed to carbon tetrachloride in ambient air and from volatilization of contaminated water during showering or bathing. Ingestion via contaminated drinking water is an important route of exposure for the general population not living in areas where carbon tetrachloride is extensively used. Dermal contact from showering or bathing has not been shown to be a significant route of exposure to carbon tetrachloride.

Most carbon tetrachloride released to the environment is expected to volatilize rapidly due to its high vapor pressure. Outdoor measurements in several areas of the United States have reported average concentrations of carbon tetrachloride in air between 0.6 and 1.0 $\mu g/m^3$ (0.1–0.16 ppb). Typical indoor concentrations in homes in several U.S. cities were about 1.0 $\mu g/m^3$ (0.16 ppb), with some values up to 9 $\mu g/m^3$ (1.4 ppb). Indoor concentrations in indoor air were thought to be higher than in outdoor air because of the presence of carbon tetrachloride in building materials or household products. The majority of domestic water supplies contain carbon tetrachloride at concentrations below 0.5 µg/L. Children are expected to be exposed to carbon tetrachloride by the same routes that affect adults. Since carbon

tetrachloride has a low affinity for adsorption onto soil and dust particles, the risk of exposure for small children from ingesting soil or dust is likely to be low. The average daily intake of carbon tetrachloride for the general population is estimated as 0.1 μg/kg/day from inhalation exposure and 0.01 μg/kg/day from ingesting drinking water containing typical low concentrations of the chemical.

See Chapter 6 for more detailed information regarding concentrations of carbon tetrachloride in environmental media.

2.2 SUMMARY OF HEALTH EFFECTS

As a volatile halogenated alkane, carbon tetrachloride has depressant effects on the central nervous system that are most significant at high exposure levels. Carbon tetrachloride also produces irritant effects on the gastrointestinal tract. Most other toxic effects of absorbed carbon tetrachloride are related to its metabolism by mixed function cytochrome P-450 oxygenases (in humans, primarily CYP2E1, but also CYP3A). The liver is the most sensitive target in exposed humans and animals, independent of the route of administration, because of the abundance of CYP2E1 and other cytochromes. The kidneys are also sensitive targets in humans and animals. There is no conclusive evidence from epidemiological studies of workers or the general population that carbon tetrachloride is carcinogenic in humans. Carbon tetrachloride has been shown to be carcinogenic in animals following chronic inhalation or oral exposure. Alcohol consumption is an important risk factor for the development of serious toxicological effects following exposure to carbon tetrachloride, since alcohol induces CYP2E1, leading to increased production of reactive metabolites. Several case reports demonstrated that when groups of individuals were accidentally exposed to carbon tetrachloride in the workplace, the individuals who were heavy consumers of alcohol developed the most serious adverse effects.

Studies in animals, combined with limited observations in humans, indicate that the principal adverse health effects associated with inhalation exposure to carbon tetrachloride are central nervous system depression, liver damage, and kidney damage. Case reports in humans and studies in animals indicate that the liver, kidney, and central nervous system are also the primary targets of toxicity following oral exposure to carbon tetrachloride; gastrointestinal irritation has been frequently noted following accidental ingestion of high doses in humans. Limited dermal data suggest that carbon tetrachloride absorbed through the skin can cause, in addition to skin irritation, gastrointestinal effects such as nausea and vomiting and neurological effects such as polyneuritis in humans, and liver damage in animals. Based on the no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL)

values identified in the animal studies, the liver appears to be the most sensitive target. Several types of liver effects have been observed in humans and laboratory animals. At lower adverse effect levels, hepatocytes accumulate lipids, resulting in cellular vacuolization and fatty degeneration. At higher exposure levels, hepatocellular necrosis (cell death), fibrosis, and cirrhosis are observed. Hepatic carcinogenicity has been observed in laboratory rodents following chronic-duration inhalation or oral exposure to carbon tetrachloride. In animal studies, kidney effects, such as tubular cell degeneration and fatty accumulation, are typically observed at higher oral doses than hepatic effects. However, in rats with chronic progressive nephropathy, hepatotoxicity and exacerbation of the severity of renal disease occurred at similar effect levels following chronic inhalation exposure. Human case reports indicate that high oral or inhalation exposures sufficient to cause renal failure (progressive uremia and electrolyte retention) may cause delayed secondary damage (edema) to the lungs. Central nervous system effects following inhalation or oral exposure include headache, weakness, lethargy, stupor, blurred vision, and coma; neurological effects are generally observed at exposure levels higher than the thresholds for hepatic or renal toxicity. High-level inhalation or oral exposure is associated with mild hematological effects, primarily anemia in humans and animals, and reduced platelet function (clotting efficiency) in animals. Suppression of immune function (reductions in IgM antibody-forming cell activity, T-cell activity, lymphocyte counts, or host resistance to bacteria) has been observed in animals exposed short-term to oral doses higher than those causing liver effects.

No studies were located regarding reproductive effects in humans after exposure to carbon tetrachloride and the available human data for developmental effects are limited to epidemiological studies of pregnancy outcomes in women exposed to carbon tetrachloride and other halogenated hydrocarbons in drinking water. These data are inadequate for establishing a causal relationship between carbon tetrachloride exposure and developmental toxicity in humans. In animals exposed by inhalation for intermediate durations, reproductive effects included decreased fertility and testicular atrophy. In developmental studies in animals exposed by inhalation or ingestion, no fetal toxicity was observed in the absence of maternal toxicity and morphological defects were not observed in offspring. However, oral doses that produced clear maternal toxicity increased fetal mortality, in some cases, complete litter loss. It is not known whether litter loss is the result of toxicity to the fetus or to the placenta.

The following sections discuss significant effects resulting from exposure to carbon tetrachloride in greater detail: hepatic, renal, neurological, and cancer.

Hepatic Effects. Hepatotoxicity is the major effect of exposure to carbon tetrachloride by any route in humans and animals and is the basis for all MRLs derived for that compound. Liver injury is detectable by clinical signs (jaundice, swollen and tender liver), biochemical alterations (elevated levels of hepatic enzymes in the blood, loss of enzymatic activities in the liver), or histological examination (fatty degeneration and necrosis of central hepatocytes, destruction of intracellular organelles, fibrosis, cirrhosis). Elevated levels of serum enzymes (alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase, and gamma glutamyl transferase) may provide evidence of hepatocellular injury in the absence of clinical signs, as was observed in workers occupationally exposed at intermediate-to-chronic durations at levels between 1.1 and 12 ppm. Degeneration or necrosis of the liver was noted in humans following acute inhalation exposure at 250 ppm or acute oral exposure at ≥110 mg/kg. In humans, acute lethal inhalation or oral exposures were associated with massive liver necrosis and steatosis. In rats, centrilobular vacuolization was observed at an acute oral dose of 20 mg/kg/day, whereas necrosis was observed at 80 mg/kg/day. Hepatic necrosis was also observed in guinea pigs following acute dermal exposure at 513 mg/cm^2. In chronic studies, significant increases in fatty change, fibrosis, and cirrhosis were observed in rats at 25 ppm; in the same study, the major non-neoplastic hepatic lesion in mice was necrosis. These species differences in hepatic effects may be related to the differential involvement of tumor necrosis factor alpha, which may facilitate necrosis, or transforming growth factor beta, which is an initiator of fibrosis.

It is widely agreed that the reason for the special sensitivity of the liver to carbon tetrachloride toxicity is the inherently high rate of metabolism of carbon tetrachloride by this tissue, presumed to be associated with the high abundance of CYP2E1, particularly concentrated in the centrilobular zone. This hypothesis was verified for mice in a study that administered 1,590 mg carbon tetrachloride/kg body weight by intraperitoneal injection to wild-type or CYP2E1 knockout mice *(cyp2e1$^{-/-}$)*. In wild-type mice expressing CYP2E1, hepatotoxicity characterized by elevated serum enzyme levels (ALT and AST) and histopathology (centrilobular parenchymal degeneration and perivenular vacuolation) was observed 24 hours after treatment with carbon tetrachloride. None of these hepatic lesions were observed in CYP2E1 knockout mice treated with the same dose. In humans, CYP2E1 is also the primary enzyme responsible for metabolizing carbon tetrachloride at environmentally relevant concentrations, but others, particularly CYP3A, are also involved at higher concentrations. The reactive metabolites (trichloromethyl free radicals) generated by the oxidation of carbon tetrachloride are believed to trigger a spectrum of hepatocellular damage. Mechanisms that appear to be involved include direct binding of reactive metabolites to cellular proteins, peroxidation of unsaturated membrane lipids, and alterations in intracellular calcium levels. Release of proteolytic enzymes from dying cells has been shown to extend

the necrosis of hepatic tissue beyond the initial site exposed to carbon tetrachloride. The outcome of any carbon tetrachloride-induced injury has been demonstrated to depend on several factors, including the induction of P-450 enzymes and the presence of antioxidants and interactions with other chemicals.

Renal Effects. Injury to the kidney is also observed in many reports of carbon tetrachloride toxicity in humans, often at the same exposure levels that cause hepatic injury. The principal clinical signs in severe cases are oliguria or anuria, with resultant azotemia and edema, leading in turn to hypertension and pulmonary edema. Cells of the proximal tubule are most clearly injured by carbon tetrachloride, probably because of high content of cytochrome P-450. Renal injury is observed in animal studies, but usually at higher doses with lesser severity than in humans. In oral exposure studies, the effect levels for kidney toxicity are generally higher than for hepatic toxicity. Some intermediate-duration inhalation bioassays in rats reported the adverse effect level for the kidney to be the same as or higher than that for the liver. In a 2-year inhalation study in F344 rats, exposure to carbon tetrachloride at hepatotoxic levels increased the severity of chronic progressive nephropathy compared to the control group.

There is some evidence that the susceptibility of the kidney to carbon tetrachloride may increase in elderly animals. Both the liver and the kidney exhibit age-related reductions in CYP-450, which would result in relatively lower production of reactive metabolites following exposure, but findings have been qualitatively different in the two organs. Reductions in CYP3A and CYP2E1 activity have been noted in the liver of elderly humans and rats. In the rat kidney, however, one isoform of CYP3A was upregulated by 50% in old (25–26-month) rats, resulting in a net 11% increase (not statistically significant) in total CYP3A in the kidney, possibly contributing to increased renal vulnerability to carbon tetrachloride. In addition to the possibility that the kidney in the elderly generates relatively more reactive metabolites following exposure, reductions in the organ content of antioxidants protecting against reactive metabolites may contribute to sensitivity. Both the liver and the kidney exhibit age-related reductions in glutathione. One study in F344 rats (the same strain used in the chronic inhalation assay) reported a significant decrease in glutathione peroxidase activity in the kidney, but not the liver of 24-month-old rats compared to 6-month-old rats, consistent with a greater sensitivity in the kidney. Although the findings outlined above are suggestive, no study has specifically demonstrated age-related changes in the renal metabolism of carbon tetrachloride.

Neurological Effects. The primary acute toxicological effect of unmetabolized carbon tetrachloride is depression of the central nervous system. Acute-duration inhalation or oral exposures in humans have resulted in neurological effects such as headache, dizziness, and weakness, and, at higher exposures,

tremor, blurred vision, drowsiness, seizures, and loss of consciousness. Immediate fatalities occur from suppression of respiratory centers. Following ingestion of single doses, autopsy findings have included neurohistopathology in the cerebellum (demyelination and Purkinje cell damage with widespread hemorrhagic infarcts), but survivors appear not to have lasting cerebellar deficits. Suppression of autonomic respiratory centers is also observed in animals exposed by inhalation. Degeneration of the optic nerve has sometimes been noted in humans and repeated inhalation exposure studies in animals, sometimes at levels lower than those causing overt signs of central nervous system depression. It is likely that the observed neurohistopathology may be related to the generation of reactive metabolites in the neural tissues of exposed animals.

Cancer. There are a few reports of cancer in people who have been exposed to carbon tetrachloride, but these data alone are not sufficient to show that carbon tetrachloride causes cancer in humans. Suggestive data in humans comes from occupational case-control studies that found positive associations between exposure to carbon tetrachloride and mortality from several types of cancer (lymphosarcoma, lymphatic leukemia, non-Hodgkin's lymphoma, or multiple myeloma). There is convincing evidence that exposure to carbon tetrachloride leads to hepatic tumors in rodents exposed by inhalation or dosed orally. The lowest cancer effect levels were observed for mice: 25 ppm by inhalation and 20 mg/kg/day orally.

Two kinds of processes appear to contribute to the carcinogenicity of carbon tetrachloride. Genotoxicity, primarily covalent binding to DNA in the liver, results from the direct binding of reactive carbon tetrachloride metabolites or lipid peroxidation products in animals exposed orally or by intraperitoneal injection. High oral doses have resulted in DNA breakage, detectable by electrophoresis. It is likely that DNA breakage following acute exposures at high levels may be secondary to liver necrosis that is characterized by the release of nucleases and other enzymes from lysosomes of degenerating hepatocytes. There is some evidence that carbon tetrachloride may also cause cancer by a nongenotoxic mechanism involving cellular regeneration. Mild hepatic necrosis stimulates cell division processes; the resulting increase in cell proliferation could result in either the replication of unrepaired DNA damage or the induction of additional errors during the replication process, both of which can produce heritable mutations that may result in an initiated preneoplastic cell.

The U.S. Department of Health and Human Services has determined that carbon tetrachloride may reasonably be anticipated to be a carcinogen. IARC has classified carbon tetrachloride in Group 2B, possibly carcinogenic to humans. EPA has determined that carbon tetrachloride is a probable human

carcinogen and derived an oral slope factor of $1.3x10^{-1}$ per (mg/kg/day). EPA is currently revising the carcinogenicity assessment for carbon tetrachloride.

2.3 MINIMAL RISK LEVELS

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for carbon tetrachloride. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

The liver is the most sensitive target organ for carbon tetrachloride toxicity by the oral and inhalation routes. Adverse effects are also observed in the kidney, which is a significant target in humans acutely exposed at high levels and in animals following chronic inhalation exposure. However, all derived MRLs for carbon tetrachloride are based on nonneoplastic hepatic effects, which occurred at lower exposure levels than effects in other target tissues in studies that quantified exposure. All derived MRLs are based on rat studies since the observed nonneoplastic hepatic effects (fatty degeneration, necrosis, fibrosis, and cirrhosis) in this species are similar to the range of histopathology observed in exposed humans. Conversely, in exposed mice, the most significant nonneoplastic features of hepatic histopathology are fatty degeneration and necrosis, but not fibrosis or cirrhosis. Thus, studies in rats would appear to be preferred as a basis for human health risk assessment for carbon tetrachloride. The MRLs for carbon tetrachloride were based on the lowest available LOAELs or the associated NOAELs (if available) in well-designed studies.

Inhalation MRLs

The database for acute-duration inhalation exposure to carbon tetrachloride includes a few studies in humans as well as studies in laboratory animals. No neurological effects were observed in six volunteers exposed to 50 ppm carbon tetrachloride for 1–3 hours, but these individuals exhibited a decrease in serum iron levels that was not observed at 10 ppm (Stewart et al. 1961); the significance of serum iron to hepatic toxicity is not clear. Single exposures of 200 ppm for ≤3 hours resulted in hepatic effects (increased serum bilirubin), renal effects (proteinuria), and gastrointestinal effects (nausea) (Barnes and Jones 1967; Norwood et al. 1950). Ethanol consumption increases the severity of carbon tetrachloride toxicity and confounds dose-response assessments from case reports. Whereas two workers experienced only mild neurological effects (headache and dizziness) after 4 hours exposure at 250 ppm, a co-worker who was a heavy consumer of alcohol died from renal insufficiency six days after a 15-minute exposure (Norwood et al. 1950); severe effects in the fatal case were noted in the liver (necrosis), kidney (oliguria, nephrosis), and lung (edema secondary to kidney failure). A NOAEL for hepatic effects was not observed in acute-duration inhalation studies in animals. Effects in rats included slight fatty degeneration of the liver at 10 ppm, increasing in extent and severity at ≥25 ppm, cirrhosis at ≥200 ppm, and parenchymatous degeneration of renal tubules in female rats treated at 400 ppm following exposure 7 hours/day for 13 days in a 17-day period (Adams et al. 1952). Mild liver effects (altered glycogen distribution, hepatocytic steatosis, hydropic degeneration, and necrosis, and elevated serum alanine aminotransferase) were observed in rats exposed at 50 ppm for 6 hours/day for 4 days (David et al. 1981). Hepatic effects (fatty degeneration and elevated serum sorbitol dehydrogenase) were also reported in rats exposed to 100 ppm 8 hours/day, 5 days/week for 2 weeks (Paustenbach et al. 1986a). Exposure for 15 minutes at 180 ppm resulted in increased alanine aminotransferase and relative liver weight in rats (Sakata et al. 1987). Effects in other organ systems following acute-duration inhalation exposure of rodents include hematological effects (increased coagulation time) at 325 ppm (Vazquez et al. 1990), developmental effects (decreased fetal body weight and crown-rump length) at 330 ppm (Schwetz et al. 1974), and neurological effects (coma, inhibition of response to electrical stimulus) at 180–1,370 ppm (Frantik et al. 1994; Sakata et al. 1987); dogs exposed to 15,000 ppm for 2–10 hours exhibited depression of the central nervous system (Von Oettingen et al. 1949). No renal effects were observed in rats exposed once or repeatedly to 100 ppm (Adams et al. 1952; Paustenbach et al. 1986b). Hepatotoxicity appears to be the critical effect of acute-duration inhalation exposure because it occurs at the lowest LOAELs in laboratory animals. The Adams et al. (1952) study identified the lowest LOAEL of 10 ppm and highest NOAEL of 5 ppm. In this study, male or female Wistar rats (2–30 of one sex/group) were exposed to carbon

tetrachloride vapor at concentrations of 0, 5, 10, 25, 50, 100, 200, or 400 ppm, 7 hours/day, 5 days/week for 173–205 days of exposures.

No MRL was established for acute-duration inhalation exposure to carbon tetrachloride because a derivation based on the most suitable data (the minimal LOAEL of 10 ppm in rats reported by Adams et al. 1952) would result in an acute-duration MRL lower than the intermediate-duration MRL. The intermediate-duration inhalation MRL of 0.03 ppm, based on a NOAEL of 5 ppm and a LOAEL of 10 ppm for liver effects (Adams et al. 1952), is expected to be protective for acute-duration inhalation exposure.

- An MRL of 0.03 ppm has been derived for intermediate-duration inhalation exposure to carbon tetrachloride.

Limited human data are available for intermediate-duration inhalation exposure to carbon tetrachloride. Effects in humans exposed intermittently included gastrointestinal effects (nausea, dyspepsia) at 20–50 ppm, depression at 40 ppm, and narcosis at 80 ppm (Elkins 1942; Heiman and Ford 1941; Kazantzis and Bomford 1960). An occupational study of hepatic effects in workers exposed for <1–>5 years indicated that serum levels of hepatic enzymes were significantly elevated only at exposures >1 ppm, but the actual durations of exposure were not reported (Tomenson et al. 1995). Interpretation of this study is also limited by the finding that the group estimated to have had the highest exposure did not show the highest levels of serum enzymes. The liver appears to be the most sensitive target in animals exposed for intermediate durations. Fatty degeneration, sometimes with increased liver weight, was observed at a LOAEL of 10 ppm in rats, mice, and guinea pigs treated 6–8 hours/day, 5 days/week for 12–36 weeks or continuously for 90 days (Adams et al. 1952; DOE 1999; Japan Bioassay Research Center 1998; Prendergast et al. 1967), and 50–100 ppm in monkeys (Adams et al. 1952; Smyth et al. 1936). Increased serum enzymes and necrosis were observed in mice at 20 ppm and hamsters at 100 ppm (DOE 1999). Exposure to higher concentrations resulted in cirrhosis in guinea pigs (25 ppm) and rats (50–270 ppm) (Adams et al. 1952; Japan Bioassay Research Center 1998; Prendergast et al. 1967; Smyth et al. 1936). In studies examining other organs, renal effects (tubular degeneration) were noted at 50–200 ppm in rats (Adams et al. 1952; Smyth et al. 1936), at 90 ppm in rats and mice (Japan Bioassay Research Center 1998), and at 200 ppm in monkeys (Smyth et al. 1936). A neurological effect (injury to sciatic and optical nerves) was noted in rats at 50 ppm (Smyth et al. 1936). Hematological effects (decreased erythrocytes, hemoglobin, hematocrit; hemolysis, increased spleen weight) were observed in rats and mice exposed to 90–270 ppm (Japan Bioassay Research Center 1998; Smyth et al. 1936). Reproductive toxicity (decreased litters, testicular atrophy) was noted at 200 ppm (Adams et al. 1952; Smyth et al.

1936). Hepatotoxicity is identified as the critical effect of intermediate-duration inhalation exposure to carbon tetrachloride since it was noted at the lowest LOAELs.

The intermediate-duration inhalation MRL for carbon tetrachloride is based on a NOAEL of 5 ppm and a LOAEL of 10 ppm for liver effects in rats identified in a study by Adams et al. 1952. In this study, Wistar rats (15/sex/group) were exposed to carbon tetrachloride vapor at concentrations of 0, 5, 10, 25, 50, 100, 200, or 400 ppm, 7 hours/day, 5 days/week for periods between 173 and 205 days. Fatty degeneration and increased liver weights were evident at concentrations of ≥10 ppm, cirrhosis occurred at ≥50 ppm, and necrosis at ≥200 ppm. Renal effects (cloudy swelling of the tubular epithelium) were first evident at ≥50 ppm, with increased kidney weights and degeneration of renal tubular epithelium evident at ≥200 ppm. Testicular atrophy was observed at ≥200 ppm. A human equivalent concentration of the identified rat NOAEL of 5 ppm ($NOAEL_{HEC}$) was calculated by multiplying the duration-adjusted rat NOAEL ($NOAEL_{ADJ}$) by the ratio of the rat and human blood:gas partition coefficients. The $NOAEL_{ADJ}$ is 0.9 ppm (5 ppm x 7 hours/24 hours x 5 days/7 days) and the blood:gas partition coefficient ratio is 1.7 (4.52/2.64). Because the ratio was greater than 1, a default value of 1 was applied, resulting in a $NOAEL_{HEC}$ of 0.9 ppm. An uncertainty factor of 30 was applied to the $NOAEL_{HEC}$ of 0.9 ppm (3 for extrapolation from animals to humans using a dosimetric adjustment and 10 for human variability).

- An MRL of 0.03 ppm has been derived for chronic-duration inhalation exposure to carbon tetrachloride.

The chronic-duration inhalation database for carbon tetrachloride includes the occupational study by Tomenson et al. (1995) and 2-year bioassays in rats and mice (Japan Bioassay Research Center 1998; Nagano et al. 1998). As discussed under the intermediate-duration MRL, elevated hepatic serum enzymes were observed in workers who had been exposed to concentrations >1 ppm for <1–5 years, but the actual durations of exposure were not reported (Tomenson et al. 1995). Interpretation of this study is also limited by the finding that the group estimated to have had the highest exposure did not show the highest levels of serum enzymes.

In the 2-year bioassays, groups of F344/DuCrj rats and BDF_1 mice (50/sex) were treated at 0, 5, 25 or 125 ppm, 6 hours/day, 5 days/week for 104 weeks (Japan Bioassay Research Center 1998; Nagano et al. 1998). Male rats exhibited increased hemosiderin deposition in the spleen at 5 ppm and above, but this effect was the result of anemia that was observed at 13 weeks, but not 104 weeks. For most observed effects, the lowest concentration of 5 ppm was a NOAEL and 25 ppm was a LOAEL: hematological

(decreased hemoglobin, hematocrit in female rats and increased extramedullary splenic hematopoeisis in mice), body weight (reduced body weight gain), renal (altered clinical chemistry values in both species, chronic progressive glomerulonephrosis in rats, protein casts in mice), and hepatic (increased liver weight and serum enzymes in both species; fibrosis, cirrhosis, severe fatty change and granulation in rats; thrombus, necrosis, and, degeneration in mice). Mice showed increased mortality from hepatic cancer at 25 ppm, rats at 125 ppm. The severity of proteinuria, but not renal histopathology, was elevated in male and female rats treated at ≥5 ppm compared to controls; this lesion was not used as the basis for MRL derivation because the severity in control rats was so high (>90% with scores of 3+ or 4+). Hepatotoxicity is selected as the critical effect of chronic-duration inhalation exposure because the severity of effects at 25 ppm was greater compared to other end points. Furthermore, selection of hepatotoxicity as the critical effect of chronic exposure is consistent with the database for intermediate-duration inhalation exposure.

The chronic-duration inhalation bioassay in rats is selected as the principal study because it provided a NOAEL of 5 ppm and a LOAEL of 25 ppm for hepatic effects (increased serum enzyme levels, liver weight, and liver histopathology) without increased mortality (Japan Bioassay Research Center 1998; Nagano et al. 1998). The duration-adjusted rat NOAEL ($NOAEL_{ADJ}$) for hepatic effects was multiplied by the ratio of the rat and human blood:gas partition coefficients to derive a human equivalent concentration of the identified chronic rat NOAEL of 5 ppm. The $NOAEL_{ADJ}$ is 0.9 ppm (5 ppm x 6 hours/24 hours x 5 days/7 days) and the blood:gas partition coefficient ratio is 1.7 (4.52/2.64). Using standard procedures, a default value of 1 was applied because the ratio was greater than 1, resulting in a chronic-duration $NOAEL_{HEC}$ of 0.9 ppm. An uncertainty factor of 30 was applied to the $NOAEL_{HEC}$ of 0.9 ppm (3 for extrapolation from animals to humans using dosimetric adjustment and 10 for human variability), resulting in a chronic-duration inhalation MRL of 0.03 ppm.

Oral MRLs

- An MRL of 0.02 mg/kg/day has been derived for acute-duration oral exposure to carbon tetrachloride.

Limited data in humans and several studies in laboratory animals are available for acute-duration oral exposure to carbon tetrachloride. In humans, hepatic toxicity (fatty accumulation, necrosis) has been noted following ingestion of single doses of carbon tetrachloride in the range of 80–180 mg/kg (Docherty and Burgess 1922; Docherty and Nicholls 1923; Phelps and Hu 1924). Single doses of 70 mg/kg had no overt neurological effect, but various neurological symptoms indicative of depression of the central

nervous system have been reported at doses between 114 and 10,800 mg/kg (Cohen 1957; Hall 1921; Leach 1922; Stevens and Forster 1953; Stewart et al. 1963). Gastrointestinal effects in humans following ingestion of single doses include nausea at ≥100 mg/kg (Ruprah et al. 1985) and vomiting and abdominal pain at 680–910 mg/kg (Hardin 1954; New et al. 1962; Smetana 1939; Umiker and Pearce 1953; von Oettingen 1964). In laboratory animals, mild hepatic effects (cytoplasmic vacuolization and increased serum enzymes) have been reported to occur following treatment with single doses of 40–80 mg/kg or repeated dosing at 5–20 mg/kg/day (Bruckner et al. 1986; Kim et al. 1990b; Korsrud et al. 1972; Smialowicz et al. 1991). No renal effects or positive results in special tests for immunological function were observed in rats following repeated administration at 5–160 mg/kg/day (Bruckner et al. 1986; Smialowicz et al. 1991). Renal effects (fatty degeneration, swelling of convoluted tubules) were observed in dogs given single doses of 3,200–6,400 mg/kg (Chandler and Chopra 1926; Gardner et al. 1925). Hepatic toxicity is selected as the critical effect of acute-duration oral exposure to carbon tetrachloride because effects were observed at the lowest effect level.

The study of Smialowicz et al. (1991) is selected as the principal study because it provides the lowest LOAEL for hepatotoxicity, the critical effect. In this study, groups of 5–6 male Fischer rats were dosed by oral gavage with 0, 5, 10, 20, or 40 mg/kg/day for 10 consecutive days and evaluated for hepatic and renal toxicity (organ weight and serum parameters and histology) as well as some immunological end points. Another set of animals was exposed at 40, 80, or 160 mg/kg/day and evaluated for additional immunological parameters. Liver toxicity was the most sensitive effect observed in this study and was dose related in severity. Centrilobular vacuolar degeneration was barely detectable in all six animals of the 5 mg/kg/day group, whereas no liver effects were observed in any of the six controls. Hepatocellular necrosis was first evident at 10 mg/kg/day. At higher doses, serum levels of ALT and AST became significantly elevated ($p<0.01$–0.05) (20 and 40 mg/kg/day), as did relative liver weight (40 mg/kg/day). No renal effects were observed at the highest dose of 40 mg/kg/day and no immunological effects were observed at doses as high as 160 mg/kg/day. The LOAEL of 5 mg/kg/day is considered to be minimal because the type and degree of hepatic histopathology was relatively mild. A total uncertainty factor of 300 was applied to the minimal LOAEL of 5 mg/kg/day (3 for the use of a minimal LOAEL, 10 for extrapolation between animals to humans, and 10 for human variability).

- An MRL of 0.007 mg/kg/day has been derived for intermediate-duration oral exposure to carbon tetrachloride.

The intermediate-duration oral toxicity database for carbon tetrachloride is somewhat limited in that no human data are available and many studies in laboratory animals restricted analysis to the liver. The

incidence and severity of hepatic effects were dose-related in animal studies. A NOAEL of 1 mg/kg and a LOAEL of 10 mg/kg was identified for significantly elevated sorbitol dehydrogenase (SDH) and mild centrilobular vacuolization in rats exposed 5 days/week for 12 weeks (Bruckner et al. 1986); at 33 mg/kg, ALT and ornithine carbamyl transferase activities were increased and cirrhosis was observed. In mice ingesting carbon tetrachloride 5 days/week for 12–13 weeks, no hepatic effects were detected at a dose of 1.2 mg/kg (Condie et al. 1986). Significant elevation in some serum enzymes (ALT, aspartate aminotransferase [AST], lactate dehydrogenase [LDH]), and mild necrosis were seen in mice at doses of 12 mg/kg and higher (Condie et al. 1986; Hayes et al. 1986). More extensive hepatic lesions (fatty accumulation, fibrosis, cirrhosis, necrosis) were noted in rats at doses of 20–25 mg/kg and higher (Allis et al. 1990; Koporec et al. 1995). At 100 mg/kg/day, hepatic effects in rats also included cytomegaly and various types of hyperplasia, which were perhaps adaptive responses to necrosis (Koporec et al. 1995). Effects in other organ systems include reduced body weight gain at doses between 33 and 100 mg/kg/day (Bruckner et al. 1986; Koporec et al. 1995) and neurological effects (increased serotonin synthesis) at 290 mg/kg/day (Bengtsson et al. 1987). No renal effects were observed in rats exposed at 33 mg/kg/day (Bruckner et al. 1986) or mice exposed at 1,200 mg/kg/day (Hayes et al. 1986). Increased mortality was observed in rats exposed at 25 mg/kg/day (Koporec et al. 1995). Cancer (hepatoma) was observed in mice treated with 20 mg/kg/day for 120 days and hamsters treated once weekly with 120 mg/kg/day for 30 weeks (Eschenbrenner and Miller 1946; Della Porta et al. 1961). Hepatotoxicity was selected as the critical effect of intermediate-duration oral exposure to carbon tetrachloride because it occurred at the lowest effect level.

The rat study of Bruckner was selected as the principal study because it provided the lowest LOAEL for the critical effect. In this study, male Sprague-Dawley rats (15–16 per group) were administered 0, 1, 10, or 33 mg/kg carbon tetrachloride by gavage in corn oil 5 days/week for 12 weeks. Blood samples were taken at biweekly intervals throughout the study for analysis of serum parameters related to liver and kidney toxicity and both organs were examined for histopathology at termination. Slightly elevated blood levels of sorbitol dehydrogenase and mild centrilobular vacuolation of the liver were observed at 10 mg/kg, but not at 1 mg/kg. Cirrhosis, extensive degenerative hepatic lesions, and significantly elevated serum enzyme levels (ornithine carbamyl transferase and alanine aminotransferase) were observed at the high dose of 33 mg/kg. No renal effects were observed at any dose. The NOAEL of 1 mg/kg was adjusted for intermittent exposure (5 days/7 days) and divided by a total uncertainty factor of 100 (10 for extrapolation between animals to humans and 10 for human variability).

No data were located on the effects of chronic-duration oral exposure in humans. Carbon tetrachloride was employed as a positive control for hepatic cancer in several chronic oral gavage bioassays in rats and mice exposed 5 days/week for 78 weeks (NCI 1976a, 1976b, 1977); serious effects were observed at the lowest tested doses. Exposure at ≥47 mg/kg reduced survival by 46% in rats because of severe hepatotoxicity. Portal cirrhosis, bile duct proliferation, and fatty accumulation were observed in more than 55% of treated rats. In the same study, survival in mice treated at doses of ≥1,250 mg/kg was reduced by ≥80% compared to controls on account of hepatic carcinogenicity. Since a no-effect level was not identified and ATSDR does not base MRLs on doses at which serious effects occur, a chronic-duration oral MRL was not derived for carbon tetrachloride.

3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of carbon tetrachloride. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between

"less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of carbon tetrachloride are indicated in Tables 3-1 and 3-2 and Figures 3-1 and 3-2. Because cancer effects could occur at lower exposure levels, Figure 3-2 also shows a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

3.2.1 Inhalation Exposure

The highest NOAEL values and all LOAEL values from each reliable study for death, and respiratory, cardiovascular, gastrointestinal, hematological, hepatic, renal, dermal, body weight, neurological, reproductive, and developmental effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.1 Death

In the past, when industrial and household use of carbon tetrachloride was still common, inhalation exposure to carbon tetrachloride resulted in a considerable number of deaths in humans (e.g., Forbes

Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
ACUTE EXPOSURE							
Death							
1	Human	15 min				250 M (1 alcoholic male died)	Norwood et al. 1950
2	Rat	8-10 hr				3000 (1/50)	Adams et al. 1952
3	Mouse	8 hr				9500 (LC50)	Svirbey et al. 1947
Systemic							
4	Human	Up to 3 hr	Hepatic		200 M (increased serum bilirubin)		Barnes and Jones 1967
			Renal		200 M (proteinuria)		
5	Human	15 min	Resp			250 M (edema)	Norwood et al. 1950
			Gastro		250 M (nausea)		
			Hepatic			250 M (severe central necrosis)	
			Renal			250 M (oliguria, nephrosis)	
6	Human	70-180 min	Cardio	50 M			Stewart et al. 1961
			Gastro	50 M			
			Hepatic	10 M	50 M (decreased serum iron)		
			Dermal	50 M			

Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
7	Rat	7 hr	Hepatic	50 M	100 M (fatty degeneration)		Adams et al. 1952
			Renal	100 M			
8	Rat	5-20 d 7 hr/d 5d/wk	Hepatic		10 M (fatty degeneration in 18 M treated 13 times over 17 d)		Adams et al. 1952
9	Rat	1d-15 wk 2d/wk 4hr/d	Hepatic			4800 M (necrosis, fibrosis, cirrhosis, mitogenic and anti-mitogenic activities)	Belyaev et al. 1992
10	Rat	4 d 6hr/d	Hepatic		50 M (steatosis, hydropic degeneration, necrosis, 2x increased alanine aminotransferase)		David et al 1981
11	Rat	2 wk 5d/wk 8hr/d or 11.5hr/d	Hepatic		100 (Fatty degeneration, increased serum sorbitol dehydrogenase)		Paustenbach et al. 1986b
			Renal	100			

Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
12	Rat	15 min	Hepatic		180 (increases 19x in alanine aminotransferase and 23% in relative liver weight)		Sakata et al. 1987
13	Rat	6-10 min/d 8 d	Hemato		325 (increased coagulation time)		Vazquez et al. 1990
Neurological							
14	Human	15 min			250 (dizziness)		Norwood et al. 1950
15	Human	70-180 min		50			Stewart et al. 1961
16	Rat (albino)	4 hr			611 M (30% inhibition of response to electrical stimulus)		Frantik et al. 1994
17	Rat	15 min				180 (coma)	Sakata et al. 1987
18	Mouse (H)	2 hr			1370 F (30% inhibition of response to electrical stimulus)		Frantik et al. 1994

Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form
19	Dog	2-10 hr					15000	(depression of central nervous system)	Von Oettingen et al. 1949
Developmental									
20	Rat	9 d Gd 6-15 7hr/d			330	(decreased fetal body weight and crown to rump length)			Schwetz et al. 1974
INTERMEDIATE EXPOSURE									
Death									
21	Monkey	6 wk 5d/wk 8hr/d					80	(1/3)	Prendergast et al. 1967
22	Rat	173-205 d 5d/wk 7hr/d					200	(9/15 male, 6/15 female)	Adams et al. 1952
23	Gn Pig	180-260d 5d/wk 7hr/d					100	(7/8 males, 4/8 females)	Adams et al. 1952
24	Gn Pig	6 wk 5d/wk 8hr/d					80	(3/15)	Prendergast et al. 1967
25	Gn Pig	90 d cont.					10	(3/15)	Prendergast et al. 1967
Systemic									
26	Human	8 hr/d intermit.	Gastro		20	(nausea)			Elkins 1942

Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
27	Human	2 mo 8hr/d 5d/wk	Gastro		50 (dyspepsia, nausea)		Kazantzis and Bomford 1960
28	Monkey	232-277d 5d/wk 7hr/d	Resp	100			Adams et al. 1952
			Cardio	100			
			Gastro	100			
			Hemato	100			
			Musc/skel	100			
			Hepatic	50	100 (slight fatty degeneration)		
			Renal	100			
29	Monkey	10.5 mo 8hr/d 5d/wk	Cardio	200			Smyth et al. 1936
			Hemato	200			
			Hepatic		50 (fatty degeneration)		
			Renal		200 (cloudy swelling of cells in convoluted tubules and loop of Henle)		
30	Rat (Fischer- 344)	12 wk 6 hr/d 5 d/wk	Hepatic	20 M	100 M (increased serum ALT, SDH; hepatic necrosis)		DOE 1999

Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form
31	Rat	173-205 d 5d/wk 7hr/d	Resp	200					Adams et al. 1952
			Cardio	200					
			Hemato	200					
			Hepatic	5[b]	10	(hepatic fatty degeneration and incr liver wt)	50	(cirrhosis)	
			Renal	100	200	(degeneration of tubular epithelium, elevated blood urea nitrogen, and increased organ weight)			
32	Rat (Fischer- 344)	13 wk 6 hr/d 5 d/wk	Hemato	30	90	(decreased hemoglobin and hematocrit; increased spleen wt in F)			Japan Bioassay Research Center 1998
			Hepatic		10	(granulation, fatty change; increased liver wt)	270	(fibrosis, cirrhosis; incr liver wt, serum enzyme incr)	
			Renal		10 M	(increased kidney weight)	270	(protein casts in M and vacuolization of tubules in F)	
			Bd Wt	270 M	810 M	(decreased bd wt)			

Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
33	Rat	6 wk 5d/wk 8hr/d	Resp	80			Prendergast et al. 1967
			Cardio	80			
			Hemato	80			
			Hepatic			80 (fatty infiltration, cirrhosis)	
			Renal	80			
34	Rat	90 d cont.	Resp	10			Prendergast et al. 1967
			Cardio	10			
			Hemato	10			
			Hepatic	1	10 (fatty degeneration)		
			Renal	10			
35	Rat	10.5 mo 8hr/d 5d/wk	Cardio	400			Smyth et al. 1936
			Hemato	50	100 (hemolysis)		
			Hepatic	50		100 (cirrhosis)	
			Renal		50 (swelling of cells in the convoluted tubules and loop of Henle)		

Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
36	Mouse (B6C3F1)	12 wk 6 hr/d 5 d/wk	Hepatic	5 M	20 M (increased serum ALT, SDH; necrosis)		DOE 1999
37	Mouse	1d-15 wk 2d/wk 4hr/d	Hepatic			4800 (necrosis, fibrosis, cirrhosis, mitogenic and anti-mitogenic activities)	Belyaev et al. 1992
38	Mouse BDF1	13 wk 6 hr/d 5 d/wk	Hemato	90 F	270 F (decr erythrocyte and hemoglobin)		Japan Bioassay Research Center 1998
			Hepatic	10 F	10 M (cytological alterations)	30 (hepatic collapse; proliferative ducts in F)	
			Bd Wt	10 M	30 M		
39	Gn Pig	4-9 mo 5d/wk 7hr/d	Hepatic	5	10 (fatty degeneration)	25 (cirrhosis)	Adams et al. 1952
40	Gn Pig	90 d cont.	Resp	10			Prendergast et al. 1967
			Cardio	10			
			Hemato	10			
			Hepatic	1	10 (fatty degeneration)		
			Renal	10			

Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
41	Hamster (Golden Syrian)	12 wk 6 hr/d 5 d/wk	Hepatic	20 M	100 M (increased serum ALT and SDH; necrosis)		DOE 1999
Neurological							
42	Human	>3 mo 8hr/d 5d/wk				80 (narcosis)	Heimann and Ford 1941
43	Human	2 mo 8hr/d 5d/wk			40 (depression)		Kazantzis and Bomford 1960
44	Monkey	232-277d 5d/wk 7hr/d		100			Adams et al. 1952
45	Rat	10.5 mo 8hr/d 5d/wk				50 (sciatic and optic nerve injury)	Smyth et al. 1936
Reproductive							
46	Rat	10.5 mo 8hr/d 5d/wk		100		200 (decreased litters)	Smyth et al. 1936
CHRONIC EXPOSURE							
Death							
47	Rat (Fischer- 344)	104 wk 6 hr/d 5 d/wk				125 (survival decreased by 86% in M and 97% in F)	Japan Bioassay Res. Ctr. 1998; Nagano et al. 1998

Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
48	Mouse (BDF1)	104 wk 6 hr/d 5 d/wk				25 (survival decreased by 29% in M and 61% in F)	Japan Bioassay Res. Ctr. 1998; Nagano et al. 1998
Systemic							
49	Rat (Fischer- 344)	104 wk 6 hr/d 5 d/wk	Hemato	5 F	25 F (decreased hemoglobin, hematocrit)		Japan Bioassay Res. Ctr. 1998; Nagano et al. 1998
			Hepatic	5[c]		25 (increased liver wt, fibrosis, cirrhosis and deposition of ceroid; increased severity of fatty change and granulation)	
			Renal	5		25 (incr marked chronic nephropathy)	
			Bd Wt	5	25 (decreased bd wt gain)		

Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
50	Mouse (BDF1)	104 wk 6 hr/d 5 d/wk	Hemato	5	25 (increased extramedullary hematopoeisis in spleen)		Japan Bioassay Res. Ctr. 1998; Nagano et al. 1998
			Hepatic	5		25 F (thrombus, necrosis) 25 (increased liver wt, degeneration, cyst, deposit of ceroid, serum enzymes, cholesterol, bilirubin)	
			Renal	5 M	25 (decreased pH and ketone bodies; protein casts, M; increased occult blood and urobilinogen, F)		
			Bd Wt			25 (marked decreased bd wt gain)	

Table 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
Cancer							
51	Rat (Fischer- 344)	104 wk 6 hr/d 5 d/wk				125 (CEL: hepatocellular adenoma in 21/50 M and 40/50 F; hepatocellular carcinoma in 32/50 M and 15/50 F)	Japan Bioassay Res. Ctr. 1998; Nagano et al. 1998
52	Mouse (BDF1)	104 wk 6 hr/d 5 d/wk				25 M (CEL: adrenal pheochromocytoma in 16/50 males)	Japan Bioassay Res. Ctr. 1998; Nagano et al. 1998
						125 F (CEL: adrenal pheochromocytoma in 22/49 females)	
						25 (CEL: hepatocellular adenoma in 27/50 males and 17/50 females; hepatocellular carcinoma in 42/50 males and 33/50 females.	

a The number corresponds to entries in Figure 3-1.

b Used to derive an intermediate-duration inhalation MRL of 0.03 ppm; the NOAEL was adjusted for intermittent exposure (7 hours/24 hours x 5 days/7 days). The duration-adjusted NOAEL of 0.9 ppm would be multiplied by the ratio of the rat and human blood:air partition coefficients (4.52/2.64) under EPA (1994) guidelines. However, as the ratio was greater than one, a default value of one was applied, resulting in a NOAEL-HEC of 0.9 ppm. The NOAEL-HEC was divided by an uncertainty factor of 30 (3 for extrapolation between animals and humans using a dosimetric adjustment and 10 for human variability).

c Used to derive a chronic-duration inhalation MRL of 0.03 ppm; the NOAEL was was adjusted for intermittent exposure (6 hours/24 hours x 5 days/7 days). The duration-adjusted NOAEL of 0.9 ppm would be multiplied by the ratio of the rat and human blood:air partition coefficients (4.52/2.64) under EPA (1994) guidelines. However, as the ratio was greater than one, a default value of one was applied, resulting in a NOAEL-HEC of 0.9 ppm. The NOAEL-HEC was divided by an uncertainty factor of 30 (3 for extrapolation between animals and humans using a dosimetric adjustment and 10 for human variability).

d Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 3-1. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

Cardio = cardiovascular; CEL = cancer effect level; cont. = continuous; d = day(s); Derm = dermal; F = female; Gastro = gastrointestinal; gd = gestation day; Gn pig = guinea pig; Hemato = hematological; hr = hour(s); incr = increased; intermit. = intermittent; LC50 = lethal concentration, 50% kill; LOAEL = Lowest-observed-adverse-effect level; M = male; min = minute(s); mo = month(s); musc/skel = musculoskeletal; NOAEL = no=observed-adverse-effect-level; ppm = parts per million; Resp = respiratory; wk = week(s).

Figure 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation

Acute (≤14 days)

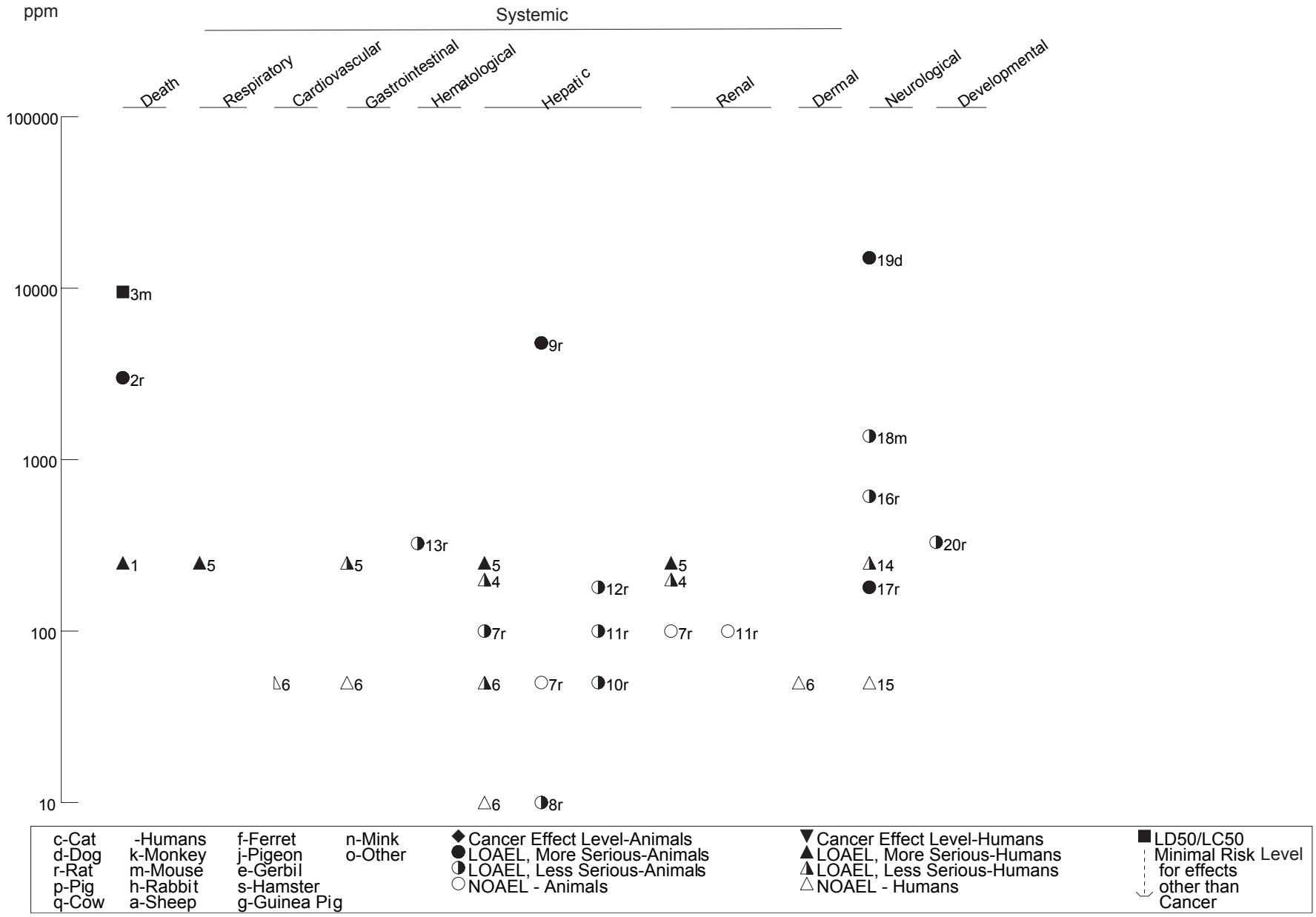


Figure 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (*Continued*)

Intermediate (15-364 days)

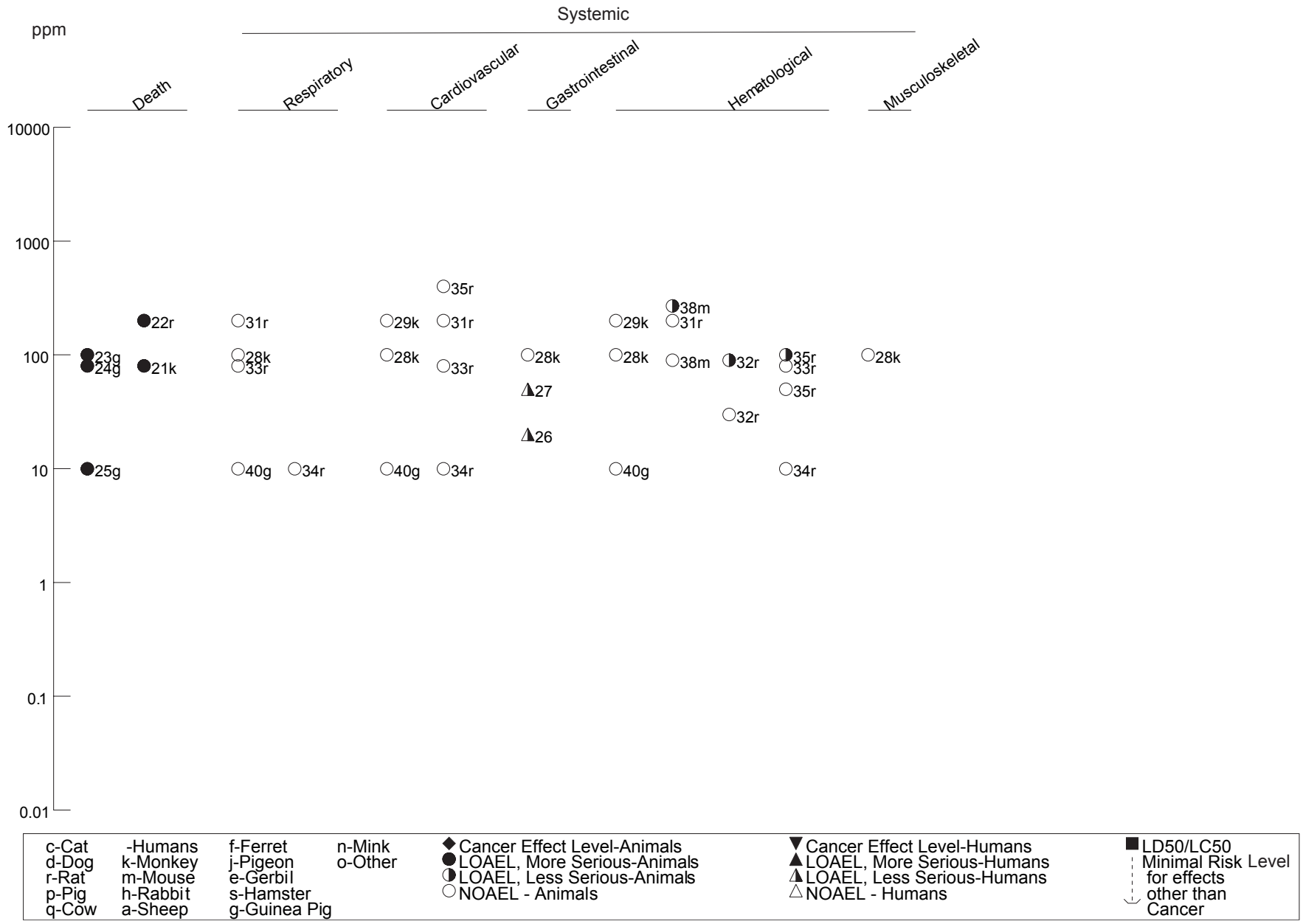


Figure 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (*Continued*)

Intermediate (15-364 days)

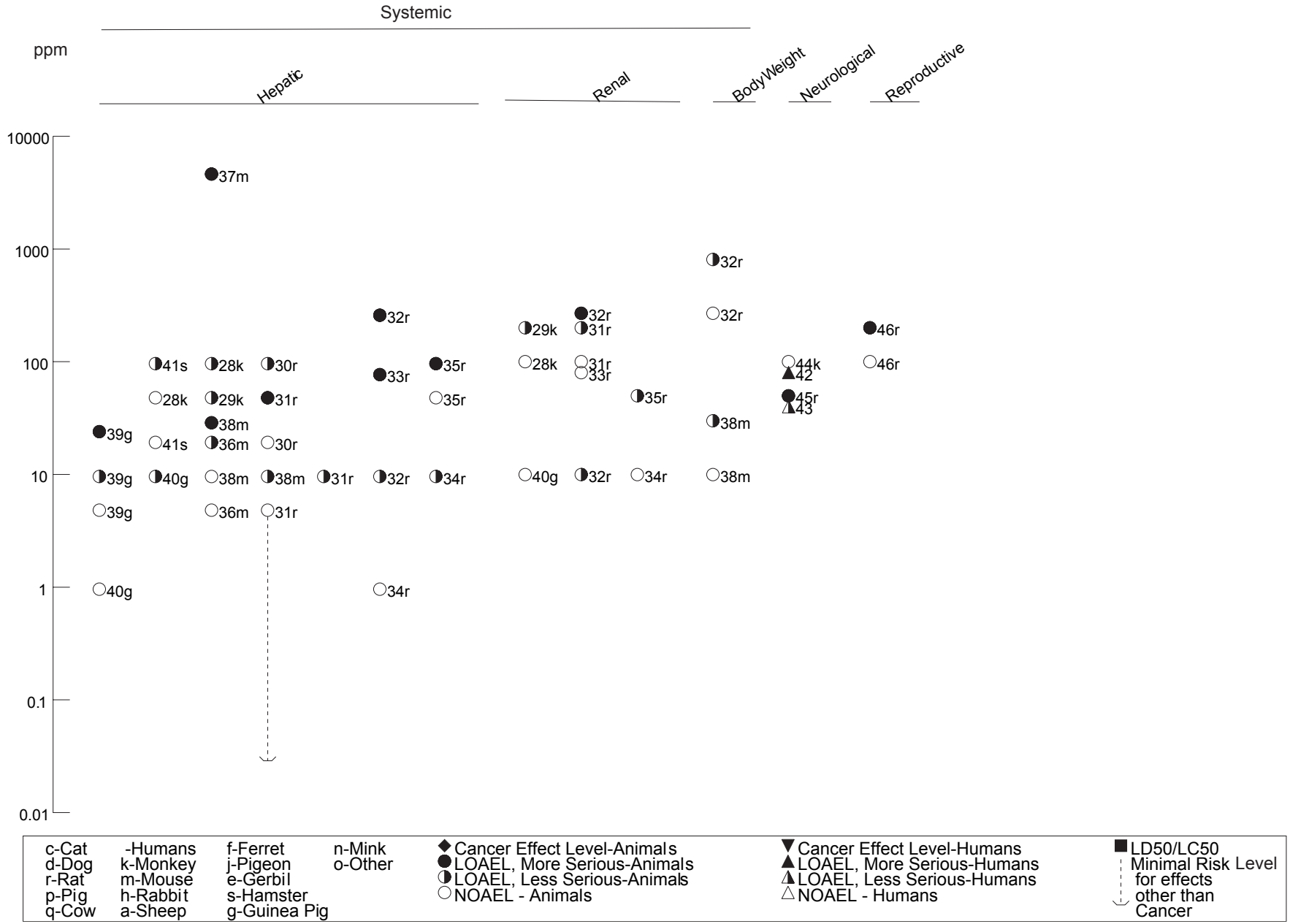
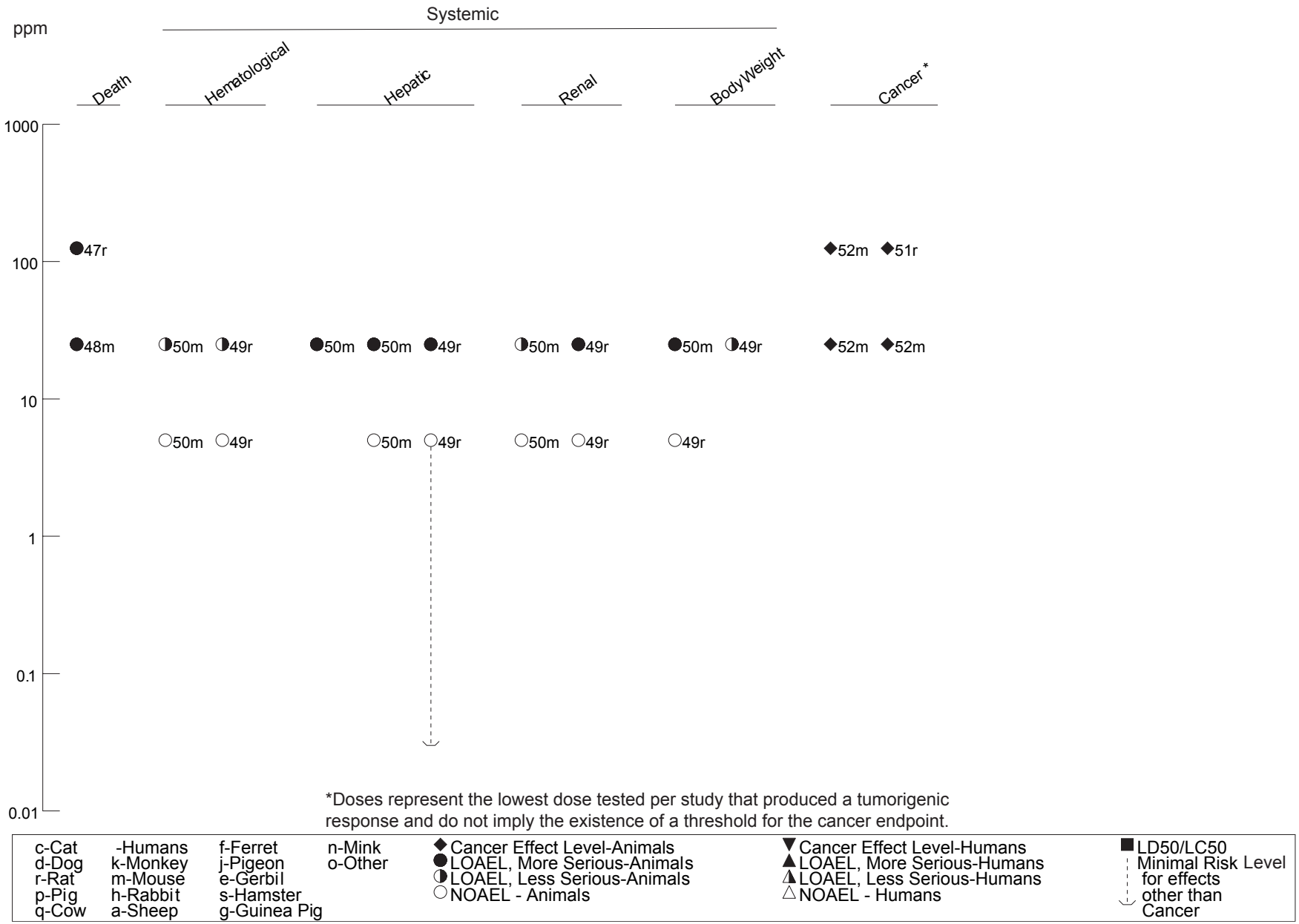


Figure 3-1 Levels of Significant Exposure to Carbon Tetrachloride - Inhalation (*Continued*)

Chronic (≥365 days)



1944; Norwood et al. 1950; Umiker and Pearce 1953). However, quantitative estimates of the exposure levels that caused death are rare; furthermore, cleaning usages often involved concurrent dermal exposure. In one case involving inhalation of carbon tetrachloride by an alcoholic, the lethal exposure level was estimated at only 250 ppm for 15 minutes (Norwood et al. 1950). Other workers (nonalcoholics) were exposed at the same level for 4 hours with no significant clinical signs other than slight headache (Norwood et al. 1950). One of three naval officers who weekly misused a carbon tetrachloride fire-extinguishing fluid as a dry cleaning agent over 3 months died of heart failure that was secondary to nephrosis-induced pulmonary edema (Forbes 1944); the man who died was a heavy consumer of alcohol. The relative exposure by inhalation or through the skin was not known, but about 3 kg carbon tetrachloride was used during 3 months.

Lethal inhalation exposure levels in animals depend on exposure duration and species. In mice, the estimated LC_{50} for an 8-hour exposure is 9,500 ppm, with no deaths in 20 animals exposed to 6,300 ppm (Svirbely et al. 1947). In rats, exposure to 7,300 ppm caused no deaths after 1.5 hours, about 50% mortality by 4–6 hours, and 100% mortality by 8 hours (Adams et al. 1952). Exposure to 3,000 ppm for 8–10 hours caused death in 1 of 50 animals. Repeated exposure to 200 ppm 7 hours/day led to increased mortality in rats after approximately 190 days (Adams et al. 1952).

All LOAEL values from each reliable study for death in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.2 Systemic Effects

No studies were located regarding musculoskeletal or endocrine effects in humans or animals after inhalation exposure to carbon tetrachloride. Studies have been conducted in both humans and animals to evaluate the respiratory, cardiovascular, hematological, hepatic, and renal effects of inhalation exposure to carbon tetrachloride. Gastrointestinal and dermal/ocular effects have been studied in humans but not in animals. These effects are discussed below. The highest NOAEL values and all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

Respiratory Effects. Pulmonary edema is a common finding in humans exposed to lethal levels of carbon tetrachloride in air. Thirteen fatal cases were reported following acute inhalation exposure in humans; exposure concentrations were not determined. Marked hemorrhagic congestion and edema were

observed in the lungs of all the victims who had been exposed for 1–6 hours (Umiker and Pearce 1953). However, these effects typically did not develop in lung until 8 days after exposure, and appeared to be secondary to severe renal injury rather than to a direct action of carbon tetrachloride on the lung. Lung appearance in the carbon tetrachloride victims was found comparable with that in cases of rapidly developing uremia occasioned by various causes of renal failure. Thus, the progressive uremia, electrolyte retention, and extracellular fluid build-up that accompanies renal failure is a likely principal cause of the observed pulmonary edema. In one fatal case, pulmonary edema secondary to renal malfunction developed following combined inhalation/dermal exposure over 3 months (Forbes 1944).

Lung injury is usually not as prominent an effect in animals exposed to carbon tetrachloride vapors as it is in humans. For example, lung injury was not observed in rats exposed to concentrations of 3,000–19,000 ppm for 7 hours, or in rats and monkeys exposed to 100 ppm for 7 hours/day, 5 days/week for 205 and 232 days, respectively (Adams et al. 1952). As it appears that lung injury is secondary to renal injury, then the absence of lung effects in animals may be because animals are also less susceptible to the renal injury produced by carbon tetrachloride than are humans.

Cardiovascular Effects. Most studies of humans exposed to carbon tetrachloride by inhalation have not detected significant evidence of cardiovascular injury, even at exposure levels sufficient to markedly injure the liver and/or kidney. Changes in blood pressure, heart rate, or right-sided cardiac dilation have sometimes, but not always, been observed (Ashe and Sailer 1942; Guild et al. 1958; Kittleson and Borden 1956; Stewart et al. 1961; Umiker and Pearce 1953), and are probably secondary either to fluid and electrolyte retention resulting from renal toxicity, or to central nervous system effects on the heart or blood vessels. Failure of the left side of the heart was the proximate cause of death in one naval officer who had a combined inhalation/dermal exposure over 3 months (Forbes 1944). The heart effect was secondary to the pulmonary edema that had developed from renal toxicity. Carbon tetrachloride also may have the potential to induce cardiac arrhythmias by sensitizing the heart to epinephrine, as has been reported for various halogenated hydrocarbon propellants (Reinhardt et al. 1971).

Similarly, except for what are likely secondary effects following acute lethal exposures, significant cardiovascular injury has not accompanied hepato- or nephrotoxic inhalation exposure to carbon tetrachloride in a variety of experimental animals (Adams et al. 1952; Prendergast et al. 1967; Smyth et al. 1936; von Oettingen et al. 1949).

Gastrointestinal Effects. One of the most common signs of exposure of humans to carbon tetrachloride is dyspepsia, with nausea, vomiting, and gastrointestinal pain (Forbes 1944; Stewart and Witts 1944). This is often one of the first clinical signs to become apparent following acute exposure (Guild et al. 1958; Norwood et al. 1950), but is also common in persons exposed for months to several years to concentrations as low as approximately 20 ppm (Elkins 1942; Smyth et al. 1936). Exposure levels of approximately 50 ppm do not cause significant dyspepsia if exposure is brief (Stewart et al. 1961), but may lead to nausea if exposure extends for several days (Kazantzis and Bomford 1960). Because inhalation exposure is unlikely to be directly irritating to the gastrointestinal tract, it is probable that these effects are secondary to effects on the autonomic nervous system (Stewart and Witts 1944).

Hematological Effects. Significant effects on the hematological system are not usually observed in humans exposed to carbon tetrachloride by inhalation (Heimann and Ford 1941; Norwood et al. 1950; Smyth et al. 1936). In some cases, moderate elevations in white cell counts are observed, perhaps in response to necrosis in the liver or kidneys. In a few cases, mild anemia is observed (Gray 1947), and may occasionally become severe (Straus 1954). A cross-sectional study of hepatic function in workers exposed <1–>5 years to carbon tetrachloride reported small (2.5–3.6%), statistically significant reductions in some hematological parameters compared to non-exposed workers, but without a dose response (Tomenson et al. 1995). At the low (<1 ppm) or medium (1–3 ppm) exposure levels, there were decreases in packed cell volume and in hemoglobin and erythrocyte count for the medium exposure group; no significant changes from controls were noted in the high exposure group (>4 ppm). The mechanism underlying anemia is not known, but it might be secondary to internal hemorrhaging as a result of decreased synthesis of clotting factors by the liver or a direct effect on bone marrow cells (Guild et al. 1958; Stevens and Forster 1953; Straus 1954). Since lipid peroxidation caused by carbon tetrachloride also affects calcium sequestration, clotting functions, which are regulated by calcium sequestration would be expected to be impaired, resulting in a tendency for internal hemorrhaging.

Similar observations have been obtained in inhalation studies in animals. Prothrombin time increased and there was lengthened activated partial thromboplastin time in rats exposed 22–40 times to 325 ppm carbon tetrachloride for 10 minutes/day, 5 days/week, indicating defective coagulation in both the extrinsic and intrinsic clotting pathways (Vazquez et al. 1990). No significant effects on hematology were detected in rats, monkeys, or guinea pigs exposed to concentrations of 10–200 ppm, 7 hours/day for periods of time up to 170 days (Adams et al. 1952; Prendergast et al. 1967). Rats exposed for 10 months to 100 ppm suffered some destruction of red blood cells, but this did not result in anemia (Smyth et al. 1936). No evidence of red blood cell hemolysis was observed at 50 ppm. Decreased hemoglobin and

hematocrit levels and increased spleen weight) at ≥90 ppm and reduced erythrocyte counts at 810 ppm were observed in male and female rats intermittently exposed to carbon tetrachloride vapor for 13 weeks (Japan Bioassay Research Center 1998); in similarly exposed mice at ≥270 ppm, reduced erythrocyte and hemoglobin counts were observed in females, and at 810 ppm, there were reductions in hematocrit in females and in hemoglobin counts in males. Significant reductions in hemoglobin and hematocrit values were observed in female rats exposed to ≥25 ppm carbon tetrachloride vapor for 6 hours/day, 5 days/week for 2 years (Japan Bioassay Research Center 1998). Splenic changes related to erythrocyte destruction included increased hemosiderin deposition in male rats at ≥5 ppm, a consequence of erythrocyte destruction observed in the 13-week studies, and increased extramedullary hematopoeisis in male and female mice at ≥25 ppm.

Hepatic Effects. Carbon tetrachloride has been known for many years to be a powerful hepatotoxic agent in humans and animals. The principal clinical signs of liver injury in humans who inhale carbon tetrachloride are swollen and tender liver, elevated levels of hepatic enzyme (aspartate aminotransferase) in the serum, elevated serum bilirubin levels and the appearance of jaundice, and decreased serum levels of proteins such as albumin and fibrinogen (Ashe and Sailer 1942; McGuire 1932; New et al. 1962; Norwood et al. 1950; Straus 1954). In cases of lethal acute or repeated exposures, autopsy generally reveals marked liver necrosis with pronounced steatosis (Forbes 1944; Jennings 1955; Markham 1967; Smetana 1939), and repeated or chronic exposure leads in some cases to fibrosis and/or cirrhosis (McDermott and Hardy 1963).

Quantitative information on the inhalation exposure levels that cause significant hepatic injury in humans is sparse. Liver necrosis was reported in one fatal case involving an alcoholic who was exposed to 250 ppm carbon tetrachloride for 15 minutes (Norwood et al. 1950). Humans exposed to concentrations of 50 ppm for 70 minutes or 10 ppm for 3 hours showed no measurable change in serum enzyme levels or urinary urobilinogen levels (Stewart et al. 1961). A slight decrease in serum iron levels occurred in two of four subjects exposed to 50 ppm for 1 hour, suggesting to the authors that minimal liver injury had occurred. However, all values were within or close to the normal range of serum iron concentrations, and there were no control subjects. Consequently, it is difficult to judge if the variations observed were treatment-related and whether they were of biological significance. No hepatic effects were observed in humans exposed to average concentrations of 80 ppm for 8 hours/day, 5 days/week for 3 months (Heimann and Ford 1941).

Occasional and slight elevations of serum bilirubin levels were seen in workers exposed for 8 hours/day for several months to many years to carbon tetrachloride concentrations ranging from 10 to 100 ppm, but no other clinical signs of injury were detected (Smyth et al. 1936). Similarly, workers exposed for up to 3 hours/day to carbon tetrachloride concentrations averaging about 200 ppm displayed small increases in serum enzyme levels and serum bilirubin levels, indicative of minimal liver damage (Barnes and Jones 1967). More recently, chronic occupational exposure of 35 male workers to <1 ppm (8 hours/day) of chlorinated solvents, primarily carbon tetrachloride and perchlorethylene, was not correlated with any significant changes in standard indicators of liver function (e.g., serum levels of protein, albumin, bilirubin, alanine and aspartate aminotransferase, alkaline phosphatase, γ-glutamyl transpeptidase, and cholesterol) (Driscoll et al. 1992). However, when workers were segregated as to having relatively higher or lower exposure, higher exposure was correlated with significantly ($p<0.03$–0.05) lower fasting serum levels of three bile acids (chenodeoxycholate, taurocholate, and total deoxycholate). This effect was in the opposite direction to what might be expected based upon oral animal data and upon serum bile acid increases reported by the same authors for a companion worker population exposed to hexachloro-butadiene or trichloroethylene. Thus, these results should be viewed with caution, especially in view of the low exposure level to carbon tetrachloride and the variable concurrent exposure to several other solvents.

A cross sectional study of hepatic function (serum enzyme levels) was conducted on 135 workers occupationally exposed to carbon tetrachloride and 276 nonexposed controls who were employed in three plants in northern England (Tomenson et al. 1995). Workers were categorized according to their duration of employment (<1 year, 1–5 years, and >5 years), but the serum enzyme results were not presented by estimated duration of exposure because statistical analysis showed that duration of exposure had no significant effect. Exposures were estimated from historical personal monitoring data for each job category, and exposure groups were categorized as low (≤1 ppm), medium (1.1–3.9 ppm), or high (≥4.0–11.9 ppm). Alcohol consumption was equivalent among groups. A comparison of exposed workers and nonexposed controls found no significant difference in blood levels of alkaline phosphatase or gamma glutamyl transferase; however, when the exposed group was subdivided by level of exposure, these values were significantly elevated in the medium exposure group. None of the exposed subjects had hepatic disease that could be attributed to exposure to carbon tetrachloride.

In animals, the hepatic effects of inhalation exposure to carbon tetrachloride are much the same as in humans: elevated serum enzyme levels, steatosis, and centrilobular necrosis progressing to fibrosis. Statistically significant doubling of serum levels of mitochondrial glutamate dehydrogenase and sorbitol

dehydrogenase was observed in rats 24 hours after a 4-hour exposure at 530 ppm and of ALT and AST at 1,460 ppm (Brondeau et al. 1983). Following 4-hour exposure at 500–2,500 ppm, fasted rats showed a 2.0–2.5-fold higher level of serum enzymes indicative of hepatic injury than fed rats (Jaeger et al. 1975); the increases in serum enzyme levels were similar in fed and fasted rats at 5,000 ppm. In rats, hypoxia (10–12% oxygen) increased the severity of hepatic injury (serum enzyme levels) compared to normal air (21% oxygen) conditions during 2–3 hour exposures to carbon tetrachloride vapor (Shen et al. 1982; Siegers et al. 1985). In rats, exposure to concentrations of 10–100 ppm, 6–7 hours/day for approximately 2 weeks generally results in mild to moderate signs of liver injury (fatty degeneration), both after short-term (roughly 2 weeks) (Adams et al. 1952; David et al. 1981; Paustenbach et al. 1986a, 1986b). Four days of exposure at 50 ppm caused elevated serum alanine aminotransferase, altered hepatic glycogen distribution (preferential accumulation in the central and pericentral zones, rather than the uniform distribution observed in controls), steatosis, hydropic degeneration, and necrosis (David et al. 1981). Short-term exposure (15 minutes/day, 2 days/week for 8 weeks) caused fibrosis in rats exposed to 180 ppm (Sakata et al. 1987). A 4-hour exposure to 4,800 ppm or higher induced centrilobular necrosis within 24 hours (Belyaev et al. 1992; Magos et al. 1982). In rats exposed for 2 hours, there were significant reductions in hepatic CYP-450 levels at ≥100 ppm and significantly elevated serum enzymes (SDH 16-fold and ALT 2-fold) at 1,000 ppm (Sanzgiri et al. 1995); the results indicate an alteration of hepatic cell function at 100 ppm, but leakage from hepatocytes at 1,000 ppm. With continued biweekly exposures at 4,800 ppm for 4 hours/day, necrotic areas were largely replaced by hepatocellular proliferation after 2–3 weeks, and then fibrosis and eventually cirrhosis (Belyaev et al. 1992). Cirrhosis along with fatty degeneration was observed in rats exposed at 200 or 400 ppm (7 hours/day, 5 days/week for 2 weeks) (Adams et al. 1952). No acute MRL was established for inhalation exposure to carbon tetrachloride because the value calculated from the most acceptable data would be the same as or lower than the intermediate-duration MRL (see Section 2.3). The intermediate-duration inhalation MRL is expected to be protective for acute-duration inhalation exposures.

The pattern of hepatic injury following intermediate-duration exposure to carbon tetrachloride is similar to that seen in acute studies. Mild to moderate liver effects (increased liver weight, fatty degeneration) were observed following intermittent exposure (6–7 hours/day, 5 days/week) at 10 ppm for 12 weeks to 6 months (Adams et al. 1952; Bogers et al. 1987; DOE 1999; Japan Bioassay Research Center 1998; Smyth et al. 1936). Similar effects were observed in rats exposed continuously at 10 ppm for 6 weeks (Prendergast et al. 1967). In 12–13-week studies, increased serum enzyme levels (AST and SDH) and necrotic injury is apparent in mice intermittently exposed at 20 ppm (DOE 1999) and in rats exposed at 90 ppm or higher (Belyaev et al. 1992; Japan Bioassay Research Center 1998). Hepatic regeneration and

proliferation were evident within 2–3 weeks of treatment at 4,800 ppm (Belyaev et al. 1992). Fibrosis and cirrhosis developed in rats following intermittent (6–7 hours/day, 5 days/week) exposure at 50 ppm for 6 months (Adams et al. 1952) or at ≥90 ppm for 12–15 weeks (Belyaev et al. 1992; DOE 1999; Japan Bioassay Research Center 1998). Although hepatic histopathology was similar in rats and mice exposed for 13 weeks, only rats developed fibrosis and cirrhosis and only mice developed collapse of the liver (Japan Bioassay Research Center 1998). Guinea pigs appear to be somewhat more sensitive to carbon tetrachloride inhalation than rats (Prendergast et al. 1967; Smyth et al. 1936), and monkeys appear to be somewhat less sensitive than guinea pigs and rats (Adams et al. 1952; Prendergast et al. 1967). Another study found mice to be more susceptible to hepatic damage (necrosis) than rats or hamsters (DOE 1999). The basis of these species differences is likely related to differences in hepatic metabolism (see Section 3.4.3). Exposure to concentrations of 1–5 ppm, 6–7 hours/day, 5 days/week for periods up to 6 months or 1 ppm continuously for 6 weeks have not been observed to cause any significant changes in liver of rats, monkeys, or guinea pigs (Adams et al. 1952; Prendergast et al. 1967). The NOAEL of 5 ppm (Adams et al. 1952) was selected as the basis for an intermediate-duration MRL for inhalation exposure to carbon tetrachloride, as described in the footnote in Table 3-1.

Plummer et al. (1990) conducted experiments that suggest that the hepatotoxicity of carbon tetrachloride administered by inhalation is proportional to the concentration x time product (Haber's rule). Rats exposed for 4 weeks at equivalent time-weighted average concentrations—either continuously (24 hours/day, 7 days/week except for two 1.5-hour breaks 2 days/week) at 16 ppm or discontinuously (6 hours/day, 5 days/week) at 87 ppm—showed identical severity of liver histopathology.

In 2-year inhalation bioassays, concentration-related hepatic effects were observed in rats and in mice following intermittent exposure (6 hours/day, 5 days/week) to carbon tetrachloride vapor (Japan Bioassay Research Center 1998; Nagano et al. 1998). Alterations in some serum hepatic biomarkers were not statistically significant in rats exposed at 5 ppm. Statistically significant increases in liver weight and serum parameters (ALT, AST, LDH, leucine aminopeptidase and gamma-glutamyl transferase, total bilirubin) were observed at ≥25 ppm in rats and mice. Hepatic lesions at ≥25 ppm included basophilic, eosinophilic, clear and mixed cell foci, deposition of ceroid, fibrosis and cirrhosis, and increased severity of fatty change and granulation. In the parallel assay in mice, statistically significant decreases in some serum parameters at 5 ppm were not considered by the authors to be biologically significant because the control values for serum chemistry parameters in males were unusually high compared to available historical control values. Hepatic degeneration, thrombus, and deposition of ceroid were evident in both sexes, and hepatic necrosis was found in female mice treated at ≥25 ppm. The NOAEL of 5 ppm for

hepatic effects in rats (Japan Bioassay Research Center 1998) was selected as the basis for a chronic-duration inhalation MRL for carbon tetrachloride, as described in a footnote in Table 3-1.

Renal Effects. Nephritis and nephrosis are very common effects in humans following inhalation exposure to carbon tetrachloride (Jennings 1955; McGuire 1932; Norwood et al. 1950). The most obvious clinical signs, developing within hours to days after exposure, are oliguria or anuria with resulting edema. In some cases, this leads to generalized uremia, and is frequently accompanied by proteinuria, hemoglobinuria, and glucosuria (Forbes 1944; Guild et al. 1958; New et al. 1962; Smetana 1939; Umiker and Pearce 1953). In fatal cases, histological examination generally reveals relatively mild degeneration of the kidney (Ashe and Sailer 1942; Forbes 1944; Gray 1947; Jennings 1955; Norwood et al. 1950). The mechanism of the injury to the kidney is not known, but Sirota (1949) reported that back-diffusion of glomerular filtrate was important in the early stages of oliguria and decreased renal blood flow contributed in the later stages of oliguria following carbon tetrachloride inhalation in humans.

The exposure levels leading to renal damage in humans have not been well defined. An increased incidence of proteinuria was reported in workers exposed to vapor concentrations of around 200 ppm (Barnes and Jones 1967), while no change was observed in urinary properties following inhalation exposure to 50 ppm for 70 minutes or 10 ppm for 3 hours (Stewart et al. 1961).

Threshold concentrations for renal injury in animals exposed by inhalation to carbon tetrachloride are sometimes higher than those for hepatic effects. Animals appear to be less sensitive to renal injury than humans, possibly because of species differences in carbon tetrachloride metabolism by the kidney. No evidence of kidney damage was observed in rats, cats, monkeys, or guinea pigs exposed for 6–8 hours/day to concentrations of 10–200 ppm for periods of time from 1 to 90 days (Adams et al. 1952; Bogers et al. 1987; Prendergast et al. 1967). In a 13-week study, 10 ppm produced increased absolute and relative kidney weights in male rats; organ weight effects and vacuolization were observed in females at 90 ppm and hyaline degeneration of the glomerulus at 810 ppm (Japan Bioassay Research Center 1998). No renal effects were noted in similarly exposed mice. Slight renal swelling was noted in rats exposed to 50 ppm for 5–10.5 months for 7–8 hours/day, 5 days/week, and in monkeys exposed to 200 ppm for 10.5 months for 7–8 hours/day, 5 days/week (Adams et al. 1952; Smyth et al. 1936). Renal tubular degeneration was apparent following exposure at 200 ppm for 7 hours/day, 5 days/week for 6 months (Adams et al. 1952).

Chronic exposure (6 hours/day, 5 days/week for 2 years) to carbon tetrachloride vapor caused renal effects in rodents, with rats being more sensitive than mice (Japan Bioassay Research Center 1998). The most sensitive renal effect in rats was a dose-related enhancement of proteinuria (scores of 4+), having a significantly ($p \leq 0.01$) higher severity in males and females treated at 5 or 25 ppm (too few animals survived at 125 ppm for statistical analysis) compared to controls; however, as the severity in controls was so high (>90% of rats with scores of 3+ or 4+), the statistical difference is not biologically significant and this end point was not used as the basis for the chronic-duration inhalation MRL. Blood urea nitrogen levels and the severity and incidence of chronic progressive nephropathy were elevated at >25 ppm in both sexes. In the parallel study in mice, protein casts in the kidney were observed in male and female mice exposed at ≥25 ppm.

Dermal Effects. Very few reports mention any effect of carbon tetrachloride inhalation on the skin. Inhalation exposure to carbon tetrachloride for several days in the workplace caused a blotchy, macular rash in one man (but not in six others) (McGuire 1932). Similarly, a hemorrhagic rash occurred in a woman exposed to carbon tetrachloride vapors for several days in the workplace (Gordon 1944), and black and blue marks were seen in a patient exposed intermittently to carbon tetrachloride vapors for several years (Straus 1954). Because observations of dermal effects are so sporadic, it is difficult to judge whether these effects are related to carbon tetrachloride exposure, or are incidental. Conceivably, they may have been secondary to reduced synthesis of blood coagulation factors resulting from carbon tetrachloride-induced hepatotoxicity. No animal studies evaluated dermal effects following inhalation exposure.

Ocular Effects. Ocular effects following inhalation exposure to carbon tetrachloride are discussed under neurological effects.

Body Weight Effects. No human and very few animal reports mention the effect of carbon tetrachloride inhalation on body weight gain. In rodents intermittently exposed to carbon tetrachloride vapor for 6 hours/day, 5 days/week for 13 weeks, body weight gain was significantly reduced by 20% in male rats exposed at 810 ppm and by 8–15% in male mice exposed at ≥30 ppm, but not significantly in female rats or mice exposed at concentrations as high as 810 ppm (Japan Bioassay Research Center 1998). In the companion 2-year study, terminal body weights were depressed by 11% in male and female rats exposed at 25 ppm and by 22–45% at 125 ppm (Japan Bioassay Research Center 1998; Nagano et al. 1998); survival at 125 ppm was too low to determine statistical significance. In mice exposed at 25 ppm

for 2 years, body weight gain at termination showed a >30% reduction in males and a >20% reduction in females relative to controls (only single male and female mice survived in the 125 ppm groups).

3.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans or animals after inhalation exposure to carbon tetrachloride.

3.2.1.4 Neurological Effects

Like many volatile halocarbons and other hydrocarbons, inhalation of carbon tetrachloride leads to rapid depression of the central nervous system. Because of its central nervous system depressant properties, carbon tetrachloride was used briefly as an anesthetic in humans, but its use was discontinued because it was less efficacious and more toxic than other anesthetics available (Hardin 1954; Stevens and Forster 1953). Depending on exposure levels, common signs of central nervous system effects include headache, giddiness, weakness, lethargy, and stupor (Cohen 1957; Stevens and Forster 1953; Stewart and Witts 1944). Effects on vision (restricted peripheral vision, amblyopia) have been observed in some cases (e.g., Forbes 1944; Johnstone 1948; Smyth et al. 1936; Wirtschafter 1933), but not in others (e.g., Stewart and Witts 1944). Sudden severe epileptiform seizure and coma occurred in a subsequently fatal case following weekly combined inhalation/dermal exposure over a period of 3 months (Forbes 1944). In several fatal cases, microscopic examination of brain tissue taken at autopsy revealed focal areas of fatty degeneration and necrosis, usually associated with congestion of cerebral blood vessels (Ashe and Sailer 1942; Cohen 1957; Stevens and Forster 1953).

Exposure levels leading to effects on the central nervous systems of humans are not precisely defined. No symptoms of lightheadedness or nausea were experienced by humans exposed to 50 ppm for 70 minutes or 10 ppm for 3 hours (Stewart et al. 1961), but nausea, headache, and giddiness were found to be common symptoms in workers exposed to carbon tetrachloride for 8 hours/day at concentrations of 20–125 ppm (Elkins 1942; Heimann and Ford 1941; Kazantzis and Bomford 1960). Dizziness has also been reported in humans following short-term exposure (15 minutes) at a higher concentration (250 ppm) (Norwood et al. 1950). This suggests that the threshold for central nervous system effects in humans is, as a conservative estimate, probably in the range of 20–50 ppm for an 8-hour workday.

Central nervous system depression is also observed in animals exposed to carbon tetrachloride vapors. Rats reportedly became inactive within 15 minutes after exposure to a concentration of 180 ppm (Sakata et al. 1987), although when compared with other studies, this concentration appears too low to be capable of inducing such an effect. Drowsiness or stupor occurred in rats exposed for 0.1–8.0 hours to 4,600 ppm, with ataxia and unconsciousness at 12,000 ppm, and death (from respiratory failure) at 19,000 ppm (Adams et al. 1952). Similarly, dogs exposed for 2–10 hours to 15,000 ppm experienced profound depression of the autonomic system, as evidenced by decreases in respiration, reflex activity, body temperature, heart rate, and blood pressure (the latter due to marked vasodilation) (von Oettingen et al. 1949). Exposure of rats, monkeys, or guinea pigs to concentrations of carbon tetrachloride up to 400 ppm, 8 hours/day, 5 days/week for over 10 months did not cause any observable effects on activity, alertness, or appetite, indicating that this level did not cause obvious central nervous system depression in animals (Smyth et al. 1936). However, histological examination of sciatic and optic nerves revealed degenerative changes in a number of animals exposed to 200–400 ppm, and in a few animals (rats) after exposure to levels as low as 50 ppm under the same exposure schedule. The changes were apparently not severe enough to impair movement or vision. Exposure to ≥5 ppm carbon tetrachloride vapor for 6 hours/day, 5 days/week for 2 years resulted in decreased absolute brain weights in male, but not female, rats, however, this effect was attributed to the overall depression in body weight, since brain weights relative to body weight were increased (Japan Bioassay Research Center 1998). Furthermore, no histopathology was detected in the brain in either sex at any concentration.

The highest NOAEL values and all LOAEL values for each reliable study for neurotoxicity in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after inhalation exposure to carbon tetrachloride.

In rats that inhaled carbon tetrachloride vapors for three generations, there was a decrease in fertility in animals exposed to concentrations of 200 ppm or higher for 8 hours/day, 5 days/week for 10.5 months (Smyth et al. 1936). Since both sexes were exposed, it was not possible to determine if this was due to effects on males, females, or both. Moderate to marked degeneration of testicular germinal epithelium has been seen in rats exposed repeatedly (7 hours/day, 5 days/week) to 200 ppm or higher for 192 days (Adams et al. 1952).

Deposition of ceroid was observed in the ovaries of mice that were exposed to 125 ppm of carbon tetrachloride vapor, 6 hours/day, 5 days/week for 2 years (Japan Bioassay Research Center 1998). At 25 ppm, absolute and relative testicular weights were elevated in male mice.

All LOAEL values for each reliable study for reproductive effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.6 Developmental Effects

No studies were located on developmental effects in humans after known inhalation exposure to carbon tetrachloride. A questionnaire-based study of 3,418 pregnant women in West Germany found no association between probable occupational exposure to carbon tetrachloride (as estimated from a job exposure matrix) and the birth of infants who were small for their gestational age (Seidler et al. 1999).

In rats, inhalation exposure to 330 or 1,000 ppm for 7 hours/day on gestational days 6–15 caused reduced feed intake by dams, maternal weight loss, and clear maternal hepatotoxicity (elevated serum ALT, relative liver weight), but no effect on conception, number of implants, or number of resorptions (Schwetz et al. 1974). There were no gross anomalies, but dose-related statistically significant reductions in fetal body weight and crown-rump length were observed. The incidence of delayed ossification of sternebrae was significantly increased at 1,000 ppm. The lowest exposure level in this study is a LOAEL for both maternal and fetal toxicity.

All LOAEL values for each reliable study for developmental toxicity in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.7 Cancer

Two case reports of liver cancer in humans suggested that previous exposure to carbon tetrachloride vapors might have contributed to the development of the cancer (Tracey and Sherlock 1968; Johnstone 1948). In the first case, a 66-year-old male died of hepatocellular carcinoma 7 years after acute intoxication with carbon tetrachloride sufficient to cause jaundice as well as vomiting and diarrhea (Tracey and Sherlock 1968). As the tumor was too extensive to make a diagnosis of cirrhosis, the report

could not rule out the man's history of moderate alcohol consumption as contributory . In the second case, a 30-year-old female died of "liver cancer" after 2–3 years of occupational exposure to carbon tetrachloride that was sufficient to produce signs of central nervous system depression. The evidence of these studies is much too weak to establish a cause-and-effect relationship between exposure to carbon tetrachloride and hepatic cancer in humans.

A number of epidemiological studies have been conducted to evaluate the association of risk of increased mortality from particular types of cancer and occupational exposure to carbon tetrachloride. Both positive and negative associations have been reported, varying with the target organ. IARC (1999) has noted that few of these studies had definitive evidence of exposure to carbon tetrachloride—generally, few positive cases were identified among exposed individuals—and that extensive exposure to other possible carcinogenic chemicals could not be excluded, and in fact, was likely. Thus, the positive associations discussed below are considered suggestive, but are not conclusive.

An analysis of cancer mortality and solvent exposure among a cohort of 6,678 active and retired male workers in the rubber industry found a significant association between age-adjusted exposure to carbon tetrachloride and lymphosarcoma (odds ratio [OR] 4.2, $p<0.05$; based on six cases) and lymphatic leukemia (OR 15.3, $p<0.001$; based on eight exposed cases) (Checkoway et al. 1984; Wilcosky et al. 1984). A retrospective cohort mortality study of 14,457 workers employed at an aircraft maintenance facility for at least 1 year during 1952–1956 included 6,737 workers who had ever been exposed to carbon tetrachloride (Blair et al. 1998; Spirtas et al. 1991). In the first study, standard mortality ratios (SMRs) for selected causes of death were calculated for workers exposed to solvents compared to the Utah death rates (Spirtas et al. 1991). A statistically significant association was found in women for deaths from non-Hodgkin's lymphoma and exposure to carbon tetrachloride (SMR 325, 95% confidence interval [CI] 119–708); excess SMRs in men for non-Hodgkin's lymphoma and in both sexes for multiple myeloma following carbon tetrachloride exposure were not statistically significant. In the follow-up study, a Poisson regression analysis was performed on cancer incidence data to evaluate the risk from exposure to carbon tetrachloride (Blair et al. 1998). Among men and women, the relative risk (RR) of mortality from non-Hodgkin's lymphoma or non-Hodgkin's lymphoma following exposure to carbon tetrachloride was not significantly increased. A study of causes of death in a cohort of 5,365 workers exposed to dry-cleaning solvents in St. Louis, Missouri, found statistically significant excesses in deaths from all cancers, esophageal cancer, and cervical cancer compared to the general U.S. population (Blair et al. 1990). The risk of esophageal cancer showed a statistically significant association with estimated cumulative exposure to dry cleaning solvents (SMR = 2.8 for the highest cumulative exposure group), but

not with level or duration of exposure. The most heavily exposed workers showed a 4-fold increased risk for cancers of the lymphatic and hematopoietic system, but the number of deaths (five) was small.

No positive association was found between likely occupational exposure to carbon tetrachloride and the risk of increased mortality from pancreatic cancer among residents of 24 states (Kernan et al. 1999), astrocytic brain tumors in workers in the petrochemical industry in three states (Heineman et al. 1994), rectal cancer in Montreal (Dumas et al. 2000), renal carcinoma in Minnesota (Dosemici et al. 1999), lung cancer among men working in a chemical plant in Texas (Bond et al. 1986), or respiratory system tumors in workers in the rubber industry (Checkoway et al. 1984; Wilcosky et al. 1984). No significantly elevated risk of cancer was detected in a cohort of 4,772 Finnish laboratory workers who were exposed to chemicals including carbon tetrachloride, but the duration of exposure was too short (<16 years) to detect cancers with longer latency periods (Kauppinen et al. 2003). A case-control study estimating the occupational exposures to some industrial chemicals among women in 24 states found a weak association between probable high-intensity exposure to carbon tetrachloride and an increased risk (OR ratio 1.21, 95% CI: 1.1–1.3) of breast cancer in Caucasian women (Cantor et al. 1995). However, a retrospective cohort study of workers in an aircraft maintenance facility found no association in woman for exposure to carbon tetrachloride and increased mortality from breast cancer (Blair et al. 1998).

Chronic exposure to carbon tetrachloride vapor induced tumors in rats and mice (Japan Bioassay Research Center 1998; Nagano et al. 1998). Following intermittent exposure for 2 years (6 hours/day, 5 days/week), significant increases in the incidences of hepatocellular adenoma and carcinoma were observed in male and female rats exposed at 125 ppm (22.3 ppm, duration adjusted) and in mice exposed at ≥25 ppm (4.5 ppm, duration adjusted). Adrenal pheochromocytomas were also induced in male mice exposed at ≥25 ppm and female mice at 125 ppm.

The carcinogenicity of carbon tetrachloride is currently undergoing reassessment by the EPA under the IRIS program, with the final report scheduled for 2006. As chronic inhalation data were not available for the earlier assessment, the EPA extrapolated oral dose-response data on liver tumor risk to yield estimates of the carcinogenic risk from inhalation exposure to carbon tetrachloride (EPA 1984). Based on the assumption that a 70-kg person breathes 20 m^3/day of air and that 40% of inhaled carbon tetrachloride is absorbed, the calculated upper-bound unit risk (the upper 95% confidence limit on the excess cancer risk associated with lifetime exposure to carbon tetrachloride at a concentration of 1 μg/m^3) is 1.5×10^{-5}. Based on this, the concentration of carbon tetrachloride in air corresponding to excess cancer risk levels of 10^{-4},

10^{-5}, 10^{-6}, and 10^{-7} are 0.001, 0.0001, 0.00001, and 0.000001 ppm, respectively. Because these are upper-bound estimates, the true risk could be lower. These values are displayed in Figure 3-1.

3.2.2 Oral Exposure

The highest NOAEL values and all LOAEL values from each reliable study for death and respiratory, cardiovascular, gastrointestinal, hematolgical, musculoskeletal, hepatic, renal, immunological/ lymphoreticular, neurological, reproductive, developmental, and cancer effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.1 Death

Ingestion of concentrated solutions of carbon tetrachloride can cause death in humans within hours to days. The principal clinical signs observed in fatal cases include gastrointestinal irritation, central nervous system depression, and cardiovascular disturbances, with death usually resulting from severe injury to kidney and/or liver (Guild et al. 1958; reviewed in von Oettingen 1964).

There is considerable variation in the doses that have been found to cause lethality, with alcohol ingestion leading to markedly increased risk. Twelve fatalities were reported following oral exposure (Umiker and Pearce 1953). In most cases, about 50–150 mL had been ingested, but one case involved only 5.3 mL (about 121 mg/kg). A review of some of the earlier literature found that ingestion of 14–20 mL (320–450 mg/kg) was fatal in the majority of cases (von Oettingen 1964). In other cases, ingestion of 2.5–15 mL (60–340 mg/kg) as a treatment for hookworm produced death in only a very small number of people out of hundreds of thousands treated, although doses as low as 1.5 mL (40 mg/kg) caused death in a few cases (Lamson et al. 1928). Two fatal cases have been reported in humans dosed with approximately 70 mg/kg (Phelps and Hu 1924).

A single dose oral LD_{50} value of approximately 13,000 mg/kg was reported for mice, and 14 daily doses of 625 mg/kg were lethal for 6 of 20 exposed male mice (Hayes et al. 1986). In rats fed carbon tetrachloride in stock diets or protein-free diets, LD_{50} values of 10,200 or 23,400 mg/kg, respectively were reported (McLean and McLean 1966). The authors attributed the difference in sensitivity in animals in this study to protein depletion, which has reportedly afforded protection against carbon tetrachloride toxicity. This may result from protein depletion-induced reduction in cytochrome P-450 synthesis, with a

Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form
ACUTE EXPOSURE								
Death								
1	Human	Once (C)				40	(lowest quantifiable dose producing death out of 6 cases)	Lamson et al. 1928
2	Human	Once				70	(death in 2/2)	Phelps and Hu 1924
3	Human	Once				120 M	(lowest quantifiable dose producing death out of 12 cases)	Umiker and Pearce 1953
4	Rat	Once (G)				10200	(LD50)	McLean and McLean 1966
5	Rat (Sprague-Dawley)	Once (G)				7500	(LD50)	Pound et al. 1973
6	Rat (Sprague-Dawley)	1 d 1-2x/d (GO)				8000	(death in 17/20)	Thakore and Mehendale 1991
7	Mouse	Once (G)				13000	(LD50)	Hayes et al. 1986
8	Mouse	14 d (G)				625 M	(death in 6/20 males)	Hayes et al. 1986

Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
9	Cat	Once (G)				400 (death in 25/36)	Chandler and Chopra 1926
Systemic							
10	Human	Once	Cardio		2500 (sinus bradycardia and arrhythmia, auricoventricular nodal rhythm, auricular fibrillation)		Conaway and Hoven 1946
			Renal		2500 (increased blood urea nitrogen)		
11	Human	Once (W)	Hepatic		110 (degeneration of hepatocytes)		Docherty and Burgess 1922
			Renal		180 (swelling of proximal convoluted tubules)		
12	Human	Once (W)	Hepatic		90 (slight fatty inflitration)		Docherty and Nicholls 1923
			Renal	90			
13	Human	Once	Renal			2700 (acute tubular necrosis, increased blood urea nitrogen, anuria, proteinuria)	Guild et al. 1958

Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
14	Human	Once	Hepatic			670 (severe necrosis; fatty deposits)	MacMahon and Weiss 1929
			Renal			670 (mild proteinuria, elevated blood urea nitrogen; kidneys swollen, fatty degeneration)	
15	Human	Once	Gastro		100 (nausea)		Ruprah et al. 1985
16	Human	1-6 d	Resp			120 (substantial hemorrhagic edema of the lung)	Umiker and Pearce 1953
17	Rat (Fischer- 344)	8-10 d 1x/d (GO)	Hepatic			280 (centrilobular necrosis, increased alkaline phosphatase and 5-nucleotidase)	Blair et al. 1991

Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
18	Rat (Sprague-Dawley)	Once (G)	Hepatic	40 M	80 M (slight vacuolization of some centrilobular hepatocytes)	160 M (increased centrilobular vacuolization with significant necrosis, substantially elevated ornithine carbamyl transferase, sorbitol deydrogenase and alanine aminotranferase)	Bruckner et al. 1986
			Renal	160 M			
19	Rat (Sprague-Dawley)	11 d 9 doses (G)	Hepatic		20 M (limited centrilobular vacuolization, moderately elevated sorbitol deydrogenase, alanine aminotranferase, ornithine carbamyl transferase)	80 M (increased centrilobular vacuolization with some limited necrosis, greatly elevated ornithine carbamyl transferase, sorbitol deydrogenase, alanine aminotranferase)	Bruckner et al. 1986
			Renal	160 M			
20	Rat (Sprague-Dawley)	Once (GW)	Hepatic		10 (increased alanine aminotransferase, sorbitol dehydrogenase, ornithine carbamyl transferase; hepatic centrilobular vacuolization)		Kim et al 1990b
21	Rat	Once (F)	Hepatic		20 M (cytoplasmic vacuolization of hepatocytes)		Korsrud et al. 1972

Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
22	Rat	Once (G)	Hepatic		80 M (decreased P-450)	1600 M (centrilobular necrosis)	Matsubara et al. 1983
23	Rat (Fischer- 344)	10 d 1x/d (GO)	Hepatic		5 M[b] (slight vacuolation)		Smialowicz et al. 1991
			Renal	40 M			
24	Rat (Fischer- 344)	once (GW)	Hepatic			80 M (necrosis; increased serum alanine aminotransferase, sorbitol dehydrogenase)	Steup et al. 1993
25	Rat (Sprague-Dawley)	Once (G)	Renal		4000 M (mitochondrial swelling in cells of proximal tubules)		Striker et al. 1968

Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
26	Rat (Sprague-Dawley)	1 d 1-2x/d (GO)	Hepatic			480 M (necrosis, vacuolation; elevated serum levels of aspartate transaminase, alanine transaminase, sorbitol dehydrogenase, decreased liver microsomal cytochrome P-450, aminopyrine demethylase, aniline hydroxylase)	Thakore and Mehendale 1991
27	Rat	Once (GO)	Hepatic	800	1600 (elevated urinary taurine) 3200 (lipid vacuoles, 96 hours post-treatment)	3200 (48 hours post-treatment: necrosis lipid vacuolation, inflammation, elevated serum taurine, elevated serum alanine and aspartate amino-transferases, reduced liver taurine)	Waterfield et al. 1991
28	Mouse	Once (G)	Hepatic	10	40 (necrosis)		Eschenbrenner and Miller 1946
29	Mouse (B6C3F1)	14 d 1x/d (GO)	Hepatic		50 F (increased relative organ weight and SGPT)		Guo et al. 2000
			Bd Wt	1000 F			

Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
30	Mouse (CD-1)	14 d (G)	Hemato		625 (decreased fibrinogen and lymphocyte levels)		Hayes et al. 1986
			Hepatic		625 (increased liver weight, elevated lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase)		
			Renal	2500			
31	Dog	Once (G)	Hepatic		3200 (centrilobular necrosis)		Chandler and Chopra 1926
			Renal		3200 (fatty degeneration)		
32	Dog	Once (G)	Hepatic	160	400 (centrilobular necrosis)		Gardner et al. 1925
			Renal		6400 (fatty accumulation in cortical tubules)		
Immuno/ Lymphoret							
33	Rat	10 d 1x/d (GO)		160			Smialowicz et al. 1991

Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
34	Mouse (BALB/c)	7 d 1x/d (GO)			500 F (suppressed T-cell activity)		Delaney et al. 1994
35	Mouse (B6C3F1)	14 d 1x/d (GO)			50 F (decreased: IgM antibody-forming cell activity per spleen and host resistance to Listeria monocytogenes)		Guo et al. 2000
Neurological							
36	Human	Once (C)		70			Hall 1921
37	Human	Once (C)		120	300 (drowsiness)		Leach 1922
38	Human	Once				4800 (narcosis)	Stevens and Forster 1953
Developmental							
39	Rat (Fischer- 344)	Gd 6-15 1x/d (GO)		25 F		50 F (total litter resorption in 5/12)	Narotsky et al. 1997a
40	Rat (Fischer- 344)	Gd 6-15 1x/d (G)		25 F	50 F (maternal piloerection and reduced body wt gain)	50 F (total litter resorption in 2/14)	Narotsky et al. 1997a

Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form
41	Rat	2-3 d (G)				1400	(total litter resorption in 11/29)	Wilson 1954
42	Mouse (B6D2F1)	Gd 1-5 or GD 6-10 or Gd 11-1 1x/d (GO)		826 F				Hamlin et al. 1993
INTERMEDIATE EXPOSURE								
Death								
43	Rat (Sprague-Dawley)	13 wk 5 d/wk (GO)				25 M	(10% mortality)	Koporec et al. 1995
44	Rat (Sprague-Dawley)	13 wk 5 d/wk (GW)				25 M	(25% mortality)	Koporec et al. 1995
Systemic								
45	Rat	12 wk (GO)	Hepatic			20	(increased serum enzymes; necrosis; cirrhosis)	Allis et al. 1990

Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
46	Rat	12 wk 5d/wk 1x/d (G)	Hepatic	1[c]	10 (substantially elevated sorbitol dehydrogenase, mild centrilobular vacuolization)	33 (substantially elevated sorbitol dehydrogenase, ornithine carbamyl transferase, alanine aminotransferase, cirrhosis)	Bruckner et al. 1986
			Renal	33			
			Bd Wt	10	33 (Bd wt gain reduced by 16%)		
47	Rat (Sprague-Dawley)	13 wk 5 d/wk (GO)	Hepatic		25 M (increases 2x in serum ALT and 10x in SDH; minimal-to-slight vacuolzation, minimal fibrosis)	100 M (necrosis, hyperplasia)	Koporec et al. 1995
			Bd Wt	25 M		100 M (Bd wt decreased 25%)	
48	Rat (Sprague-Dawley)	13 wk 5 d/wk (GW)	Hepatic		25 M (increases 2 x in serum ALT and 10 x in SDH; vacuolation, fibrosis)	100 M (necrosis, hyperplasia)	Koporec et al. 1995
			Bd Wt	25 M		100 M (Bd wt decr 25%)	
49	Mouse	90 d 5d/wk (G)	Hepatic	1.2	12 (elevated alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase; mild necrosis)		Condie et al. 1986

Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
50	Mouse	120 d (G)	Hepatic	80			Eschenbrenner and Miller 1946
51	Mouse	90 d (G)	Hemato	1200			Hayes et al. 1986
			Hepatic		12 (centrilobular necrosis, elevated lactate dehydrogenase, alanine aminotransferase, asparte aminotransferase, and alkaline phosphatase)		
			Renal	1200			
52	Dog (Beagle)	28 d 1 x/d (C)	Hepatic		797 (incr serum ALT and OCT; vacuolization, single-cell necrosis)		Litchfield and Gartland 1974
53	Dog (Beagle)	8 wk 1 x/d (C)	Hepatic	32 F			Litchfield and Gartland 1974
Neurological							
54	Rat	1x/wk 10 wk (G)			290 M (increased serotonin synthesis)		Bengtsson et al. 1987
Cancer							
55	Mouse	120 d (G)				20 (CEL: hepatoma)	Eschenbrenner and Miller 1946

Table 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
56	Hamster	30 wk 1x/wk (GO)				120 (CEL: hepatoma)	Della Porta et al. 1961
CHRONIC EXPOSURE							
Death							
57	Rat (Osborne-Mendel)	78 wk 5d/wk (G)				94 M (survival at 110 wks decr by 46%)	NCI 1976
58	Mouse	78 wk 5d/wk (G)				1250 (survival decr by 80%)	NCI 1976
Systemic							
59	Rat (Osborne-Mendel)	78 wk 5d/wk (G)	Hepatic			47 M (cirrhosis, bile duct proliferation, fatty accumulatioin)	NCI 1976
Cancer							
60	Rat	78 wk 5d/wk (G)				47 M (CEL: hepatocellular carcinomas)	NCI 1976
61	Mouse (B6C3F1)	78 wk 5d/wk (G)				1250 (CEL: 100% with hepatic carcinoma)	NCI 1976

a The number corresponds to entries in Figure 3-2.

b Used to derive an acute-duration oral MRL of 0.02 mg/kg/day; the minimal LOAEL was divided by an uncertainty factor of 300 (3 for the use of a minimal LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

c Used to derive an intermediate-duration oral MRL of 0.007 mg/kg/day; the NOAEL was first adjusted for intermittent exposure (5 days/7 days) and divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

(C) = capsule; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); F = female; (F) = feed; (G) = gavage; (GO) = gavage in oil; Gastro = gastrointestinal; (GW) = gavage in water; Hemato = hematological; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; mg/kg/day = milligrams per kilograms per day; NOAEL = no-observed-adverse-effect level; Resp = respiratory; (W) = water; wk = week(s); x = time(s); yr = year(s)

Figure 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral

Acute (≤14 days)

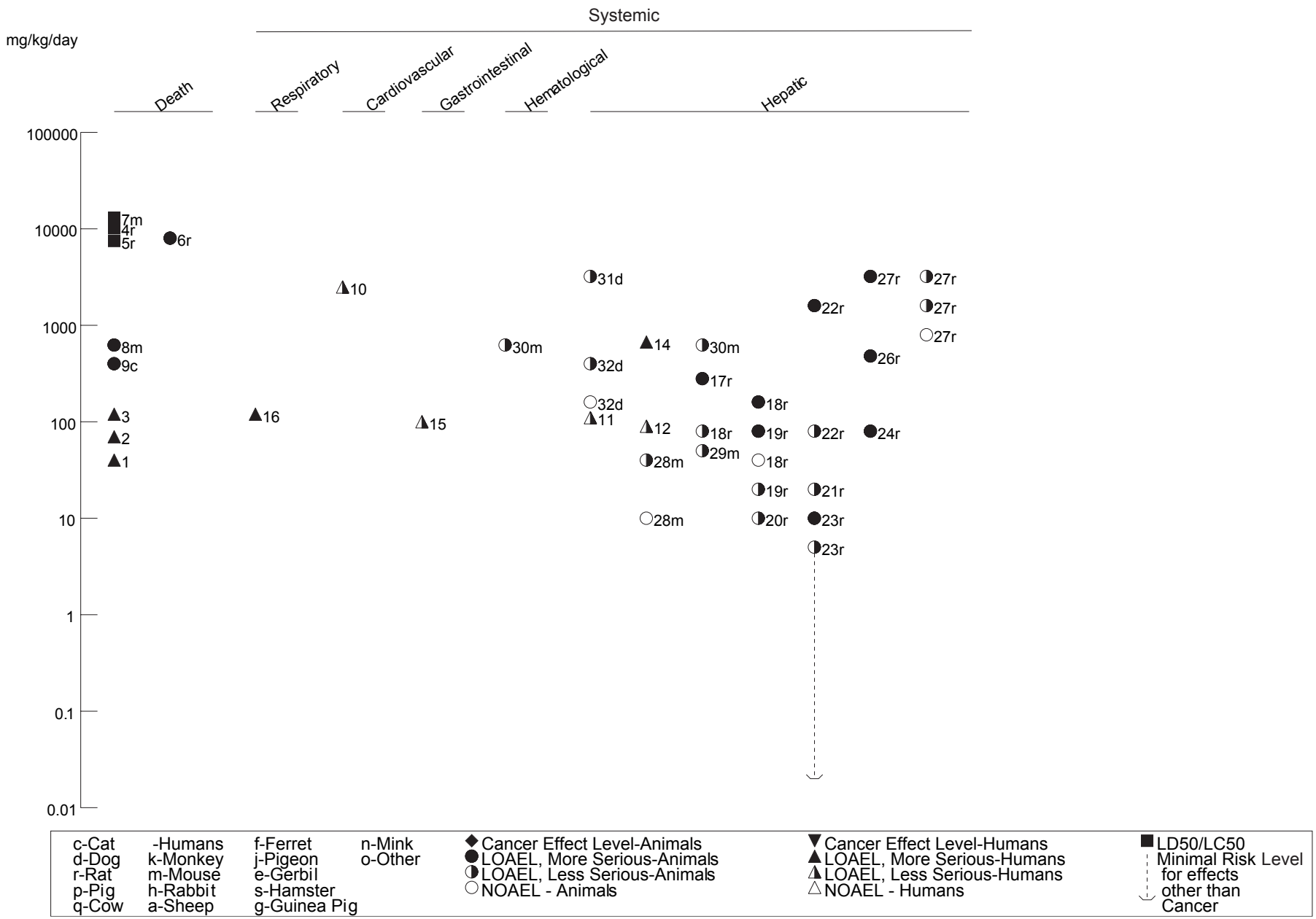


Figure 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (*Continued*)

Acute (≤14 days)

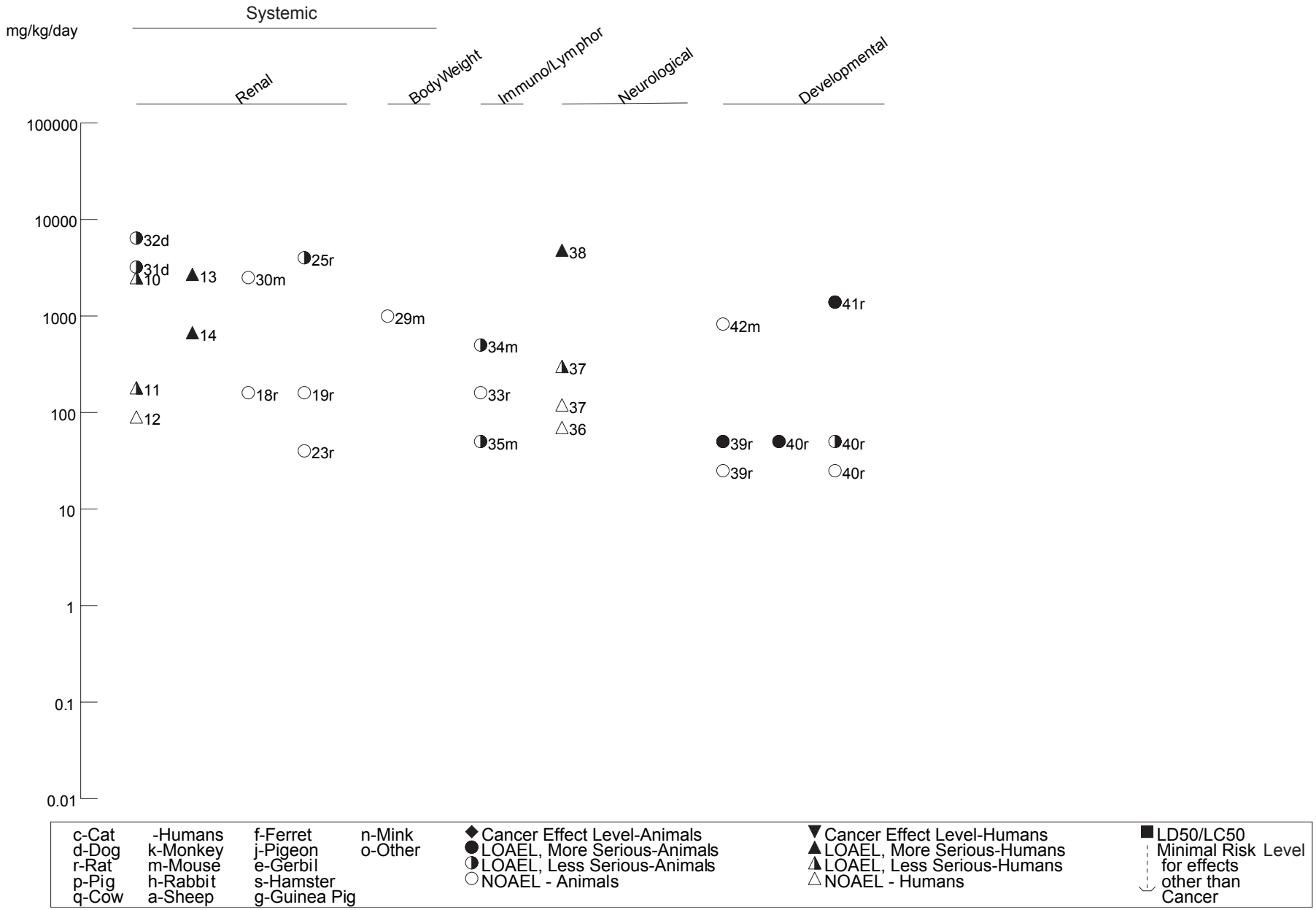


Figure 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (*Continued*)

Intermediate (15-364 days)

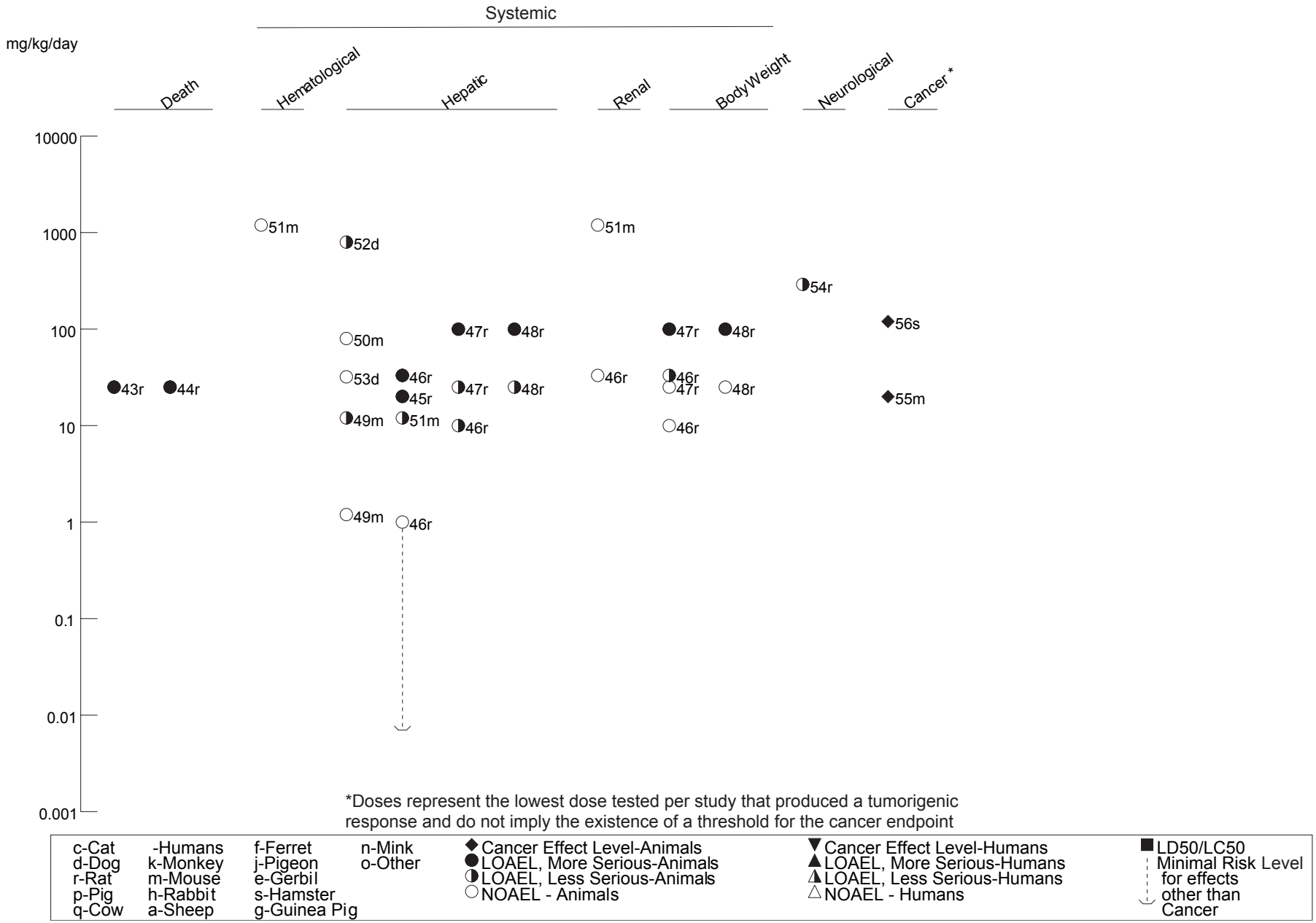
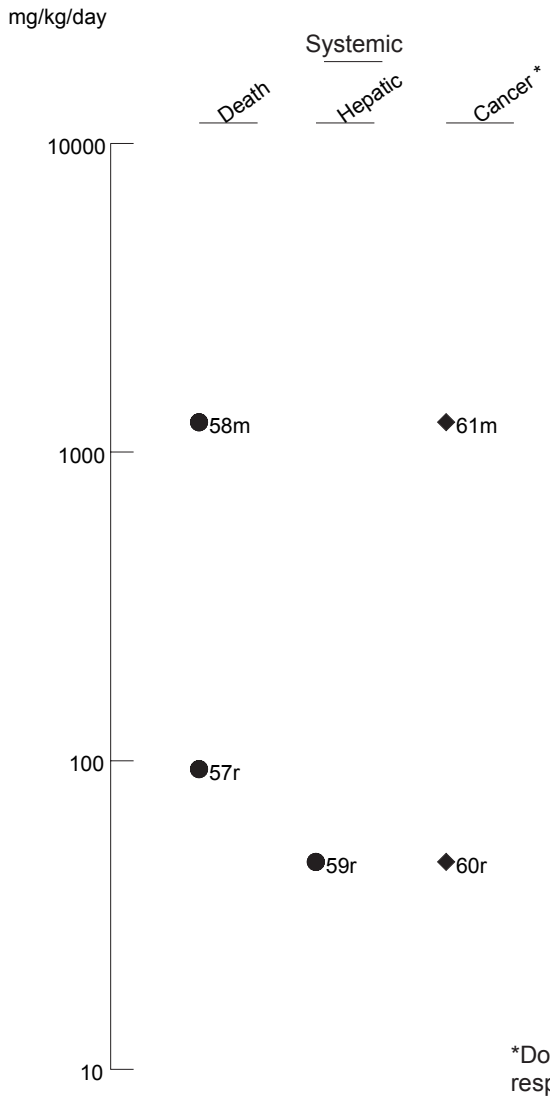


Figure 3-2 Levels of Significant Exposure to Carbon Tetrachloride - Oral (*Continued*)

Chronic (≥365 days)



*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint

c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level for effects other than Cancer
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◭ LOAEL, Less Serious-Humans	
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	
q-Cow	a-Sheep	g-Guinea Pig				

consequent diminished metabolic activation of carbon tetrachloride to toxic metabolites. In other studies using rats, an LD_{50} value of approximately 7,500 mg/kg was reported (Pound et al. 1973), while 17/20 animals were killed within 14 days of a single oral gavage exposure to 8,000 mg/kg (Thakore and Mehendale 1991). Doses as low as 400 mg/kg have resulted in the death of cats (Chandler and Chopra 1926).

One study reported a vehicle effect on mortality in rats repeatedly dosed on weekdays with carbon tetrachloride for 13 weeks (Koporec et al. 1995). When the vehicle was corn oil, mortality was 45% at the high dose of 100 mg/kg/day (average daily dose of 17.8 mg/kg/day) and 10% at the low dose of 25 mg/kg/day (average daily dose of 17.8 mg/kg/day); there were no deaths in the control group. When administration was as an aqueous emulsion in 1% Emulphor, the respective mortalities were 75 and 25%. As hepatic effects were the same for the equivalent dose groups, it is not known whether effects on other organ systems (not evaluated) were associated with the vehicle effects on mortality.

In rats exposed to ≥47 mg/kg of carbon tetrachloride 5 days/week for 78 weeks and observed for an additional period, survival at 110 weeks was reduced by 46% compared to controls because of hepatotoxicity (NCI 1976a, 1976b). In the same study, survival in mice treated at doses of ≥1,250 mg/kg was reduced by ≥80% compared to controls on account of hepatic carcinogenicity.

All LOAEL values for each reliable study for death in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.2 Systemic Effects

No studies were located regarding endocrine, dermal, ocular, or metabolic effects in humans or animals after oral exposure to carbon tetrachloride. Studies have been conducted in humans and animals to evaluate the respiratory, cardiovascular, hematological, or hepatic effects. Gastrointestinal and renal effects have been evaluated in humans, and musculoskeletal effects have been noted in animals. These effects are discussed below. The highest NOAEL values and all LOAEL values for each reliable study for systemic effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

Respiratory Effects. A number of human fatalities have been reported following ingestion of carbon tetrachloride (Umiker and Pearce 1953). Edema and hemorrhage of the lung were common autopsy

findings. Injury to the lung usually did not become apparent until 8 days or longer after poisoning, and the effects on the lung were essentially the same as observed in cases of uremia due to other causes. This suggests that the late-developing edema and hemorrhagic injury to lung is secondary to severe kidney injury.

In animals, acute oral exposure to doses of 4,000 mg/kg has been observed to cause respiratory edema, atelectasis, and hemorrhage (Gould and Smuckler 1971). This is accompanied by marked disruption of subcellular structure in most pulmonary cell types, including granular pneumocytes, capillary endothelial cells, and Clara cells (Boyd et al. 1980; Gould and Smuckler 1971; Hollinger 1982). It has been shown that Clara cells were most severely injured because they are the most active in metabolic activation of carbon tetrachloride. Injury to capillary endothelial cells is dose-dependent, with increased release of cellular enzymes occurring at doses as low as 160 mg/kg (Hollinger 1982). No studies of respiratory effects following longer-term oral exposure were located.

Cardiovascular Effects. Effects of carbon tetrachloride ingestion on the cardiovascular system have not been the subject of extensive investigation. Most studies in humans have not detected significant gross or histopathological changes in heart tissue at dose levels that cause marked hepatic and renal damage (Leach 1922; MacMahon and Weiss 1929). Electrocardiographic changes (sinus arrhythmia, QRS complex splintering, elevated S-T_4 and P-R intervals) suggestive of myocardial injury were seen in a man who ingested several mouthfuls of carbon tetrachloride, but these appeared to be fully reversible (Conaway and Hoven 1946).

The few animal studies located appear to be in general agreement with the human findings (Gardner et al. 1925; Korsrud et al. 1972). Effects of carbon tetrachloride ingestion on blood pressure are sometimes observed, but these are likely secondary to effects on the central nervous system, or to effects on fluid and electrolyte balance following renal injury.

Gastrointestinal Effects. Humans who ingest oral doses in excess of 30 or 40 mL (680–910 mg/kg) frequently experience nausea, vomiting, and abdominal pain (Hardin 1954; New et al. 1962; Smetana 1939; Umiker and Pearce 1953; von Oettingen 1964). Nausea has been reported after an oral dose of as little as 100 mg/kg (Ruprah et al. 1985). These effects could be the direct result of irritation of the gastrointestinal tract caused by the high dose or secondary to effects on the central nervous system. Oral doses of 3–5 mL (70–110 mg/kg) were widely used in the past for the treatment of hookworms with only mild gastrointestinal distress (Hall 1921; Leach 1922).

No studies were located regarding gastrointestinal effects in animals after oral exposure to carbon tetrachloride.

Hematological Effects. Oral exposure to carbon tetrachloride has not been reported to have substantial direct hematological effects in humans or animals. Focal hemorrhagic lesions and mild anemia are sometimes observed in humans who have ingested carbon tetrachloride (Guild et al. 1958; Stewart et al. 1963), but this is likely due to decreased hepatic synthesis and/or secretion of clotting factors.

Only one study was identified that examined the hematological effects of carbon tetrachloride in animals. Intermediate oral exposure of mice to carbon tetrachloride did not result in any consistently significant hematological changes (Hayes et al. 1986).

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans after oral exposure to carbon tetrachloride.

Only a single animal oral study was located that described effects on skeletal muscles, but as it employed co-treatment with phenobarbital to enhance the development of hepatotoxicity (inflammation, necrosis and fibrosis), it is omitted from the LSE table. Male rats were exposed once per week by gavage to carbon tetrachloride doses of approximately 260–1,300 mg/kg/day, for either 3 or 10 weeks (Weber et al. 1992). Histological examination of various muscle tissues revealed no evidence of necrosis or inflammation, a finding supported by normal plasma levels of albumin, creatinine, creatinine phosphokinase, and urea nitrogen. However, muscle atrophy was observed that was apparently selective for fast glycolytic fibers, but not fast or slow oxidative fibers. This was shown to result from increased protein catabolism, and not from decreased protein synthesis. Although the mechanisms are not clearly understood, this muscle effect may be secondary to induced hepatic damage. This conclusion was partially inferred from the observed complete lack of myocyte necrosis, the fiber selectivity of the effect, the absence of enhanced catabolism in muscle exposed directly *in vitro* to 10-fold higher concentrations of carbon tetrachloride, the elimination of disuse atrophy as a factor, and the correlation of this effect with only liver inflammation and necrosis, not cirrhosis (a condition which has been associated in humans with a negative nitrogen balance).

Hepatic Effects. Ingestion of carbon tetrachloride can lead to marked hepatotoxicity. In most reports involving humans, exposure has involved ingestion of several mouthfuls or more (probably 500 mg/kg or higher). Typical clinical signs of hepatic damage in such patients include a swollen liver, along with elevated serum levels of hepatic enzymes and decreased serum levels of liver-synthesized proteins (e.g., albumin, fibrinogen). In cases of death (usually occurring within 1–15 days), typical histological findings include fat accumulation, hepatic degeneration, and moderate to severe centrilobular necrosis; hepatitis was also diagnosed (Ashe and Sailer 1942; Jennings 1955; MacMahon and Weiss 1929; Umiker and Pearce 1953).

Single oral doses of 3–5 mL (70–110 mg/kg) were widely used in the past for treatment of hookworm, and ingestion of this dose resulted in clinical signs of liver injury in only a small number of cases (Hardin 1954; Lamson et al. 1928). Single doses of 4–8 mL (90–180 mg/kg) were found to result in fat accumulation in liver in several individuals (Docherty and Burgess 1922; Docherty and Nicholls 1923), and doses of only 1 mL (child) and 3 mL (adult) (approximately 80 mg/kg) have resulted in hepatic necrosis and death in a few cases (Phelps and Hu 1924). These results are indicative of differential susceptibility to carbon tetrachloride in humans. Certain confounding variables (age) may have been contributing factors to lethality at lower dose levels. One of the two cases involved a 5-year-old child, while the second report involved an adult; however, factors that may have increased susceptibility to the compound in this case could not be determined (Phelps and Hu 1924). No studies were located regarding the effects of longer-term or chronic oral exposure in humans to carbon tetrachloride.

The hepatotoxic effects of carbon tetrachloride have been widely studied in animals. Indeed, carbon tetrachloride is used as a model chemical in many laboratory investigations of the basic mechanism of action of hepatotoxic chemicals. Oral exposure to carbon tetrachloride has been observed to result in a wide spectrum of adverse effects on the liver, the most prominent of which are destruction of the smooth and rough endoplasmic reticulum and its associated enzyme activities (Reynolds and Yee 1968), inhibition of protein synthesis (Lutz and Shires 1978), impaired secretion of triglycerides with resultant fat accumulation (Recknagel and Ghoshal 1966; Recknagel and Glende 1973; Waterfield et al. 1991), centrilobular necrosis (Blair et al. 1991; Reynolds and Yee 1968; Waterfield et al. 1991), and eventually fibrosis and cirrhosis (Allis et al. 1990; Bruckner et al. 1986;). Hepatic injury, as indicated by regenerative proliferation following carbon tetrachloride ingestion, initially affects the peri-portal zone and spreads in a dose-dependent fashion to the perivenous-to-midlobular zones over time (Lee et al. 1998). In rats treated with 7,970 mg/kg, peak increases were observed in serum levels of ALT at 24 hours and AST at 48 hours and increased activity of DNA-synthesizing enzymes (thymidine kinase and

thymidylate synthetase) was observed at 48–72 hours (Nakata et al. 1975). These results reflect the initial leakage of enzymes from damaged hepatocytes followed by cell regeneration. Similar observations have been seen in dosed mice, in which hepatic necrosis was observed during the first 24 hours after dosing and hepatic mitotic activity was noted by 48 hours (Eschenbrenner and Miller 1946).

Although the occurrence of these effects has been confirmed in a very large number of studies, only a few investigations have focused on the dose-dependency of hepatic injury. After a single oral dose of 1,600 mg/kg to rats, urinary taurine levels were significantly increased ($p<0.01$–0.05) within 24 hours and liver weight was reduced (Waterfield et al. 1991). During the first 48 hours after a higher dose (3,200 mg/kg), first liver, then serum, and finally urinary levels of taurine were elevated. Similar effects, as well as reduced hepatic microsome levels of cytochrome P-450, aminopyrine demethylase, and aniline hydroxylase, were observed in rats after a single oral dose of 480 mg/kg/day (Thakore and Mehendale 1991). These effects were much more severe after 8,000 mg/kg, a dose found to be lethal within 14 days for most animals. Additionally, the liver evidenced necrosis, lipid vacuolation, and inflammation, and serum alanine and aspartate amino transferase levels were elevated. Strain differences in sensitivity have been identified (Magos et al. 1982). Twenty hours after a single dose, slight centrilobular damage was observed at 600 mg/kg in Fisher rats, but at 1,200 mg/kg in Porton-Wistar rats; the dose required to elevate serum ALT 10-fold over background was 610 mg/kg for Fisher rats, but 2,000 mg/kg for Porton-Wistar rats. These results were consistent with the higher background levels of serum ALT in Fisher rats, twice that of Porton-Wistar rats (Magos et al. 1982); this study is omitted from the LSE table because exact group sizes were not reported. Single oral doses of only 40–80 mg/kg have also been observed to produce liver injury in rats and mice (Bruckner et al. 1986; Eschenbrenner and Miller 1946; Steup et al. 1993). In rats receiving 80 mg/kg, elevated serum ALT and SDH were observed as early as 3 hours after administration and histopathology (glycogen depletion and focal necrosis) by 6 hours (Steup et al. 1993); recovery was largely complete by 72 hours. When exposures are continued for 10–11 days, doses of 5–40 mg/kg/day produced mild signs of liver change, while 80 mg/kg/day caused clear hepatic injury (Bruckner et al. 1986; Smialowicz et al. 1991). The LOAEL of 5 mg/kg/day from the study by Smialowicz et al. (1991) has been employed to calculate an acute-duration oral MRL of 0.02 mg/kg/day, as described in the footnote on Table 3-2. At this dose, the earliest sign (vacuolar degeneration) of hepatocyte toxicity was just detectable. The severity of this hepatocellular injury with accompanying necrosis increased in a dose-related manner from 10 to 40 mg/kg/day.

The incidence and severity of hepatic effects were dose-related in intermediate-duration oral exposure studies in animals. In rodents ingesting carbon tetrachloride 5 days/week for 12–13 weeks, no hepatic

effects were detected at doses of 1–1.2 mg/kg (Bruckner et al. 1986; Condie et al. 1986). At 10 mg/kg, mild centrilobular vacuolization and elevated serum levels of sorbitol dehydrogenase were observed in rats (Bruckner et al. 1986). Significant elevation in some serum enzymes (ALT, AST, lactate dehydrogenase) and mild necrosis were seen in mice at doses of 12 mg/kg and higher (Condie et al. 1986; Hayes et al. 1986). More extensive hepatic lesions (fatty accumulation, fibrosis, cirrhosis, necrosis) were noted in rats at doses of 20–33 mg/kg (Allis et al. 1990; Bruckner et al. 1986; Koporec et al. 1995). At 100 mg/kg/day, hepatic effects in rats also included cytomegaly and various types of hyperplasia (Koporec et al. 1995). The vehicle used to administer carbon tetrachloride modifies the observed effect levels. The incidence and severity of hepatic lesions was increased when carbon tetrachloride was administered in an aqueous emulsion (in 1% Emulphor) compared to administration in corn oil (Koporec et al. 1995). Dogs appear to be less vulnerable to the hepatic effects of carbon tetrachloride than rodents. In female dogs given 32 mg/kg/day for 8 weeks, there was no liver histopathology and no significant increase in serum enzymes (Litchfield and Gartland 1974). Male and female dogs that received 797 mg/kg/day for 4 weeks exhibited liver histopathology (centrilobular fatty vacuolization sometimes with single cell necrosis); the severity of hepatic lesions in individual dogs was correlated with the level of increases in serum enzyme levels (ALT and ornithine carbamyl transferase). Based on the NOAEL of 1 mg/kg in rats that was reported by Bruckner et al. (1986), an intermediate oral MRL of 0.007 mg/kg/day was calculated as described in the footnote in Table 3-2.

A no-effect level for hepatic effects has not been determined in chronic-duration oral exposure studies in animals. Alumot et al. (1976) reported no significant effects on serum enzyme levels or hepatic fat content of rats exposed to nominal doses of approximately 11–14 mg/kg/day for 2 years. However, the doses in this study cannot be reliably estimated because of uncertainty regarding the method of exposure; diets were fumigated with carbon tetrachloride vapor for 2 days prior to use and the degree of loss from evaporation is not known. Although the gradual intake from the diet would be expected to result in less hepatic toxicity than the same daily amount delivered as a single oral bolus (see, for example, Bruckner et al. 1986 discussed above), this study does not provide definitive information on threshold levels for oral exposure to carbon tetrachloride. The only other chronic-duration oral information comes from chronic gavage bioassays by the NCI (1976a, 1976b) on chloroform and trichloroethylene in which carbon tetrachloride was employed as a positive control for hepatic carcinogenicity. Rats treated with doses as low as 47 mg/kg, 5 days/week for 78 weeks exhibited severe hepatotoxicity, the most prominant effects being portal cirrhosis in 58%, bile duct proliferation in 62%, and fatty accumulation in 58% of treated animals; fibrosis, necrosis, and regenerating nodules were observed less frequently. The main hepatic effect in mice was carcinogenicity, discussed in Section 3.2.2.7. No chronic-duration oral MRL was

derived for carbon tetrachloride because the lowest tested doses in well-conducted bioassays caused severe hepatic toxicity. Under ATSDR guidance, MRLs are not derived on dose levels causing severe effects.

Renal Effects. Nephritis is a common finding in fatal cases of carbon tetrachloride ingestion in humans (Umiker and Pearce 1953), and renal failure may contribute to death in many cases (Gosselin et al. 1976; von Oettingen 1964). Typically, clinical signs of renal dysfunction (oliguria or anuria, albuminuria, proteinuria, elevated blood urea nitrogen edema, hypertension) tend to develop within 1–6 days after exposure, somewhat later than the appearance of hepatic injury (Conaway and Hoven 1946; Guild et al. 1958; Kluwe 1981; MacMahon and Weiss 1929; Smetana 1939; Umiker and Pearce 1953). In nonfatal cases, renal function usually returns to normal within several weeks (Guild et al. 1958; Kluwe 1981; Smetana 1939). Histological changes in the kidney are observed primarily in the proximal tubular epithelium, where cells become swollen and granular, with moderate to severe necrosis (Docherty and Burgess 1922; Guild et al. 1958; MacMahon and Weiss 1929; Smetana 1939).

Studies in animals confirm that the kidney is a target tissue for carbon tetrachloride, although in rodents, the kidney is much less sensitive than the liver to carbon tetrachloride. Doses of 4,000 mg/kg resulted in swollen and pale kidneys in rats within 2 days, with morphological changes present primarily in proximal tubular epithelial cells. All histological and functional signs of renal injury were fully reversible within 5 days (Striker et al. 1968). Fatty degeneration of the kidney has been observed in dogs after a single dose of 3,200 mg/kg (Chandler and Chopra 1926) and swelling of the convoluted tubules after 6,400 mg/kg (Gardner et al. 1925). Exposure of rats to 160 mg/kg/day for about 10 days did not induce adverse renal effects (Bruckner et al. 1986; Smialowicz et al. 1991), nor did 12 weeks exposure to 33 mg/kg/day, 5 days/week (Bruckner et al. 1986). Only marginal indication of kidney injury was detected in mice exposed to doses of 2,500 mg/kg/day for 14 days or 1,200 mg/kg/day for 90 days (Hayes et al. 1986). It should be recalled that these doses result in marked hepatotoxicity.

Body Weight Effects. Reduced body weight gain has been observed in rats orally dosed with carbon tetrachloride 5 days/week for 90 days. Terminal body weights were 16% lower than controls in rats dosed with 33 mg/kg (Bruckner et al. 1986) and 25% lower in rats dosed with 100 mg/kg (Koporec et al. 1995).

3.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans after oral exposure to carbon tetrachloride.

Studies in rodents have shown significant suppression of immune function following exposure to carbon tetrachloride. Exposure of female mice to carbon tetrachloride at 500 mg/kg/day for 7 consecutive days suppressed the T-cell-dependent humoral responses to sheep red blood cells (SRBC) (Delaney et al. 1994). The effect was mediated by an increase in serum levels of transforming growth factor beta-1 (TGF-beta-1), which occurred 24–48 hours after exposure in single-dose experiments (at 250–500 mg/kg, but not 50 mg/kg). Exposure of rats to carbon tetrachloride (up to 160 mg/kg/day for 10 days) by gavage did not alter the primary antibody response to SRBC, lymphoproliferative responses to mitogen or mixed leukocytes, natural killer cell activity, or cytotoxic T-lymphocyte responses; also, spleen and thymus weights were comparable to controls (Smialowicz et al. 1991). In female mice that were given daily gavage doses between 50 and 500 mg/kg/day for 14 days (sufficient for hepatotoxicity), the T-cell-dependent humoral response to SRBC was suppressed at ≥50 mg/kg/day, serum anti-SRBC IgM titers were reduced at 100 mg/kg/day, and the absolute number and percentage of $CD4^{+}CD8^{-}$ T-cells per spleen was reduced at 500 mg/kg/day (Guo et al. 2000). Exposure had no effect on the mixed leukocyte response to allogenic spleen cells, or the activities of cytotoxic T-lymphocytes or natural killer (NK) cells. In this study, exposure to carbon tetrachloride also decreased host resistance to *Streptococcus pneumoniae* and *Listeria monocytogenes*, with the effective dose dependent on the magnitude of the challenge. In rats exposed twice weekly for 4–12 weeks to 3,688 mg/kg/day, there was histologic evidence of hemorrhage, hemosiderin deposition, and lymphocyte depletion in the pancreaticoduodenal lymph node (Doi et al. 1991), an effect that may be secondary to induced hepatic damage.

The highest NOAEL values and all LOAEL values for each reliable study of immunological and lymphoreticular effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.4 Neurological Effects

Ingestion of carbon tetrachloride frequently results in marked depression of the central nervous system. Neurological signs in humans include headache, vertigo, weakness, blurred vision, lethargy, and coma, sometimes accompanied by tremor and parasthesias. Mental confusion and disorientation tend to appear

later. These symptoms have been reported in people who ingested single oral doses of carbon tetrachloride ranging from 5 to 473 mL (approximately 114–10,800 mg/kg) (Cohen 1957; Leach 1922; Stevens and Forster 1953; Stewart et al. 1963). The onset of initial effects is very rapid, and is likely the result of direct narcotic action on the central nervous system, similar to other anesthetic halocarbons. Recovery from the depressant effects generally appears to be complete (Stevens and Forster 1953; Stewart et al. 1963), although in some fatal cases, histological examination of the brain has revealed patchy pontine necrosis, demyelination, and Purkinje cell damage, with widespread hemorrhagic infarcts (Cohen 1957). Single oral doses of 70 or 120 mg/kg have been reported to be without significant neurological effect (Hall 1921; Leach 1922).

Only one animal study was located that specifically reported neurological effects other than those that typically attend acute high-dose exposure (e.g., lethargy, coma, related cardiac effects of arrhythmia, and blood pressure changes). When rats pretreated with phenobarbitol received weekly doses of carbon tetrachloride for 10 weeks (initially 289 mg/kg/day, increasing to a maximum of approximately 1,600 mg/kg/day according to body weight gain), a condition of diffuse micronodular liver cirrhosis was induced (Bengtsson et al. 1987). This was accompanied by significantly increased synthesis of the neurotransmitter serotonin in all six areas of the brain that were monitored. Serotonin levels were not, however, reliably correlated with any abnormal open-field behavior, which was used as an indicator of the possible portal-systemic encephalopathy that may accompany liver failure.

The highest NOAEL values and all LOAEL values for each reliable study for neurological effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to carbon tetrachloride.

Rats (males and females) ingested carbon tetrachloride in their food for 5–6 weeks (Alumot et al. 1976). No effects were noted on most reproductive parameters monitored (percent conception, percent with litters, mean litter size, mean body weight of offspring at birth and at weaning). An increase in neonatal mortality was observed in the low dose group (about 6 mg/kg/day), but not in the high dose group (about 15 mg/kg/day). The authors concluded that this response was not treatment related, and that these doses of carbon tetrachloride had no adverse effect on reproduction.

The highest NOAEL values for reproductive effects in rats after chronic exposure are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.6 Developmental Effects

An epidemiological study was conducted using birth outcome and drinking water exposure databases from a four-county area in northern New Jersey (Bove et al. 1992a, 1992b, 1995). The cross-sectional study of data from 75 out of 146 towns spanned the period 1985–1988 and evaluated the entire study population of 80,938 singleton live births and 599 singleton fetal deaths. Estimated carbon tetrachloride concentrations in the drinking water of >1 ppb were associated with the following adverse developmental outcomes (odds ratio, 95% confidence interval, significance): full-term birth weight <2,500 g (2.26, 1.41–3.6, $p<0.001$), small for gestational age (1.35, 1.03–1.8, $p<0.03$), and neural tube defects (5.39, 1.31–22.2, $p<0.025$). However, the weight/size effects were also associated with trihalomethanes that were present in the drinking water at larger concentrations than carbon tetrachloride and the neural tube defects were based on a total of two cases in the group exposed to carbon tetrachloride. Methodological limitations of the study may have resulted in chance, missed, or under- or overestimated associations. As acknowledged by the authors, inhalation and/or dermal exposure through bathing and showering could be at least as significant as the oral exposure. Although these studies suggest a causative role for carbon tetrachloride in the generation of certain adverse developmental outcomes, issues that could beneficially be addressed in the future include better-defined exposure levels (these levels appear to be rather low for a causative agent) and the potential for such effects to be the result of complex mixture exposure.

A case-control study of selected congenital malformations and maternal residential proximity to NPL sites in California between 1989 and 1991 did not find an increased risk of conotruncal heart defects or oral cleft defects associated with sites containing carbon tetrachloride (Croen et al. 1997).

No teratogenic effects (morphological anomalies) were reported in rats following maternal oral exposure to carbon tetrachloride, but total resorption of fetuses was reported at maternally toxic doses. Doses of 1,400 mg/kg/day during gestation caused marked maternal toxicity in rats, and total resorption of fetuses in some animals, but no adverse effects in surviving litters (Wilson 1954). In rats treated with carbon tetrachloride by gavage in corn oil or an aqueous vehicle (Emulphor EL-620) on gestational days 6–15, no maternal or developmental toxicity occurred at a dose of 25 mg/kg/day (Narotsky et al. 1997a). Total loss of some litters and clinical signs of toxicity (piloerection and reduced body weight gain) occurred in

dams treated with ≥50 mg/kg/day. Effects were slightly more severe when the vehicle was corn oil (5/12 litters resorbed) than when an aqueous vehicle was used (2/24 litters resorbed).

Temporal variations during gestation in sensitivity to carbon tetrachloride were reported in rats. When pregnant rats were given a single dose of 150 mg/kg carbon tetrachloride on gestational day 6, 7, 8, 10, or 12, the incidences of full litter loss ranged between 36 and 72% during gestation days 6–10 (maximal day 8) and 0% on day 12 compared to 4% for the controls (Narotsky et al. 1997b). The authors concluded that gestational days 6–10 represented a critical period of vulnerability to carbon tetrachloride in rats. Dams later found to have had full litter resorption exhibited bloody vaginal discharges within 24 hours of dosing. No additional developmental toxicity was reported in surviving litters. Offspring were not evaluated for possible neurobehavioral deficits.

Mice appear to be less vulnerable than rats to carbon tetrachloride. When pregnant mice were given oral doses as high as 826 mg/kg/day on five consecutive days (the preimplantation period [gestation days 1–5], or organogenesis periods [gestation days 6–10 or 11–15]), there were no overt signs of maternal toxicity and no adverse effects on survival, growth or development during the fetal and postnatal stages (Hamlin et al. 1993).

The highest NOAEL value for developmental effects in rats after acute exposure is recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.7 Cancer

No studies were located regarding carcinogenic effects in humans after oral exposure to carbon tetrachloride.

Studies in animals (rats, hamsters, and several strains of mice) provide convincing evidence that ingestion of carbon tetrachloride increases the risk of liver cancer (Andervont 1958; Della Porta et al. 1961; Edwards 1941; Edwards and Dalton 1942; Edwards et al. 1942; Eschenbrenner and Miller 1944, 1946; NCI 1976a). In general, carbon tetrachloride-induced liver tumors were either hepatomas or hepatocellular carcinomas that appeared after exposure periods of only 10–30 weeks (Edwards 1941; Eschenbrenner and Miller 1944; NCI 1976a). For example, daily oral doses as low as 20 mg/kg produced hepatic tumors in mice exposed for 120 days (Eschenbrenner and Miller 1946). In most cases, the incidence of hepatic tumors was very high (75–100%) in exposed animals. In each of these studies, the

carbon tetrachloride was administered daily by single bolus gavage. As noted in the discussion of oral hepatic effects, such a dosing regimen may exacerbate cancer effects relative to those that might be observed under conditions of food or drinking water exposure. Based on these studies, both IARC (1987) and EPA (IRIS 1993) have concluded there is sufficient evidence that carbon tetrachloride is carcinogenic in experimental animals, and that it is possibly or probably carcinogenic in humans.

The EPA (1984) reviewed the available information on the carcinogenic effects of carbon tetrachloride following oral exposure, and concluded that the studies by Della Porta et al. (1961) in hamsters, Edwards et al. (1942) in mice, and NCI (1976a, 1976b, 1977; Weisburger 1977) in rats and mice had adequate dose-response data to allow quantitative estimation of the unit cancer risk (the excess risk of cancer associated with lifetime ingestion of water containing 1 μg/L, assuming intake of 2 L/day by a 70-kg person). Since each study was judged to have some limitations, no one study was selected as the basis for the risk calculation. Rather, calculations were performed for all four data sets, and the geometric mean of these estimates was taken to be the most appropriate value. These calculations are summarized in Table 3-3. Because of the uncertainty in the data and in the calculations, the EPA identified the geometric mean of the upper 95% confidence limit (3.7×10^{-6}) as the preferred estimate of unit cancer risk.

Based on this value, the upper-bound lifetime risk from ingestion of 1 μg/kg/day of carbon tetrachloride is 1.3×10^{-4}, and the daily intake levels associated with lifetime risks of 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} are 0.77, 0.077, 0.0077, and 0.00077 μg/kg/day, respectively.

Because these are based on upper-bound estimates, the true risk could be lower. These values, along with doses of carbon tetrachloride that have been observed to cause cancer in animals, are presented in Figure 3-2.

3.2.3 Dermal Exposure

The highest NOAEL values and all LOAEL values from each reliable study for death and hepatic and dermal effects in each species and duration category are recorded in Table 3-4.

Table 3-3. Summary of Carcinogenic Unit Risk Calculations for Oral Exposure to Carbon Tetrachloride[a]

		Unit cancer risk[b]	
Reference	Species	Best estimate	Upper 95% confidence limit
Della Porta et al. (1961)	Hamster	2.5×10^{-5}	3.4×10^{-5}
Edwards et al. (1942)	Mouse	7.1×10^{-6}	9.4×10^{-6}
NCI (1976)	Mouse	1.4×10^{-6}	1.8×10^{-6}
NCI (1976)	Rat	1.9×10^{-7}	3.1×10^{-7}
	Geometric Mean	2.5×10^{-6}	3.7×10^{-6}

[a]Source: EPA 1984
[b]The estimated probability of cancer in a 70-kg person ingesting 2 L/day of water containing 1 µg/L of carbon tetrachloride for a lifetime

Table 3-4 Levels of Significant Exposure to Carbon Tetrachloride - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL Less Serious		LOAEL Serious		Reference Chemical Form
ACUTE EXPOSURE								
Death								
Gn Pig	Once 24 hr					15000 mg/kg	(LD50, 24 hours)	Roudabush et al. 1965
Gn Pig	Once contact for 5 d					260 mg/cm²	(5/20)	Wahlberg and Boman 1979
Rabbit	Once 24 hr					15000 mg/kg	(LD50, 24 hours)	Roudabush et al. 1965
Systemic								
Gn Pig	Once 15 min-16 hr	Hepatic		513 mg/cm²	(hydropic changes, slight necrosis)			Kronevi et al. 1979
		Dermal		513 mg/cm²	(karyopynosis, spongiosis, perinuclear edema)			
Gn Pig	Once 24 hr	Dermal		120 mg/kg/day	(primary irritation)			Roudabush et al. 1965
Rabbit	Once 24 hr	Dermal		120 mg/kg/day	(primary irritation)			Roudabush et al. 1965

cm = centimeters; d = day(s); Derm = dermal; Gn pig = Guinea pig; hr = hour(s); LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; mg/kg/day = milligrams per kilograms per day; NOAEL = no-observed-adverse-effect level

3.2.3.1 Death

One of three naval officers who weekly misused a carbon tetrachloride fire-extinguishing fluid as a dry cleaning agent over 3 months died of heart failure that was secondary to nephrosis-induced pulmonary edema (Forbes 1944); the man who died was a heavy consumer of alcohol. It is likely that the individual was exposed by inhalation as well as dermally, but the actual intakes were not known; 4 pints of the fluid (equivalent to 3 kg carbon tetrachloride) were used during the time period.

3.2.3.2 Systemic Effects

No studies were located regarding hematological, musculoskeletal, endocrine, or ocular effects after dermal exposure of humans or animals to carbon tetrachloride. Respiratory, cardiovascular, gastrointestinal, hepatic, renal, ocular, and dermal effects were reported in humans. Hepatic and dermal effects were also seen in animals. These effects are discussed below. The LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 3-4.

Respiratory Effects. Pulmonary edema that developed in one fatal case of combined weekly inhalation/dermal exposure over 3 months appeared to be secondary to renal malfunction (Forbes 1944).

Cardiovascular Effects. Failure of the left side of the heart was the proximate cause of death in one case repeatedly exposed dermally and by inhalation over 3 months (Forbes 1944). The heart effect developed secondary to nephrosis-induced pulmonary edema.

Gastrointestinal Effects. There are case reports of three humans who experienced gastrointestinal symptoms, including nausea and vomiting, after dermal application of carbon tetrachloride-based lotion (Perez et al. 1987). No quantitative estimate of the amount of carbon tetrachloride applied or absorbed was provided. Three individuals who were repeatedly exposed dermally and by inhalation over 3 months experiences anorexia and vomiting (Forbes 1944).

No studies were located regarding gastrointestinal effects in animals after dermal exposure to carbon tetrachloride.

Hepatic Effects. Liver injury, characterized by an elevated serum enzyme (alanine aminotransferase level), was described in case reports of three humans after dermal application of carbon tetrachloride (Perez et al. 1987). In the absence of quantitative estimates of the amount of carbon tetrachloride applied or absorbed, NOAEL and LOAEL values cannot be determined. Severe centrilobular necrosis was found at autopsy following the death of one of three naval officers who were exposed by inhalation and dermally over 3 months (Forbes 1944); none of the exposed men exhibited jaundice. Actual exposure levels could not be determined.

Hydropic changes and isolated necrotic areas were reported in the liver of guinea pigs 16 hours after dermal contact with 513 mg/cm^2 of carbon tetrachloride (1.0 mL placed in a sealed enclosure covering a sealed enclosure covering a 3.1 cm^2 area of clipped skin) (Kronevi et al. 1979).

Renal Effects. Acute renal failure, as evident by anuria and azoturia, was reported in three case reports of humans after dermal application of carbon tetrachloride-based lotion (Perez et al. 1987). The usefulness of this finding is limited by the lack of data concerning the amount of carbon tetrachloride applied or absorbed. Albuminuria and uremia developed in three naval officers exposed weekly by inhalation and dermally to an unknown amount of carbon tetrachloride over 3 months (Forbes 1944). The one fatality, an alcoholic, showed signs of albuminous degeneration of the convoluted tubules and Bowman's capsule and granular casts in the distal convoluted tubules.

No studies were located regarding renal effects in animals after dermal exposure to carbon tetrachloride.

Dermal Effects. In humans, direct dermal contact with undiluted carbon tetrachloride causes a mild burning sensation with mild erythema (Stewart and Dodd 1964). Some individuals appear to be hypersensitive, developing marked swelling, itching, and blisters following dermal contact (Taylor 1925).

Similar effects of dermal contact with carbon tetrachloride have been described in animals. A dose of 124 mg/cm^2 carbon tetrachloride produced moderate primary irritation within 24 hours when applied occluded to the intact or abraded skin of rabbits or guinea pigs, with irritation scores of 2.2–4.1 on skin (Roudabush et al. 1965). Direct dermal contact of guinea pigs with liquid carbon tetrachloride (occluded; 513 mg/cm^2) caused degenerative changes in epidermal cells and marked intercellular edema or spongiosis (Kronevi et al. 1979). These effects became apparent within 15 minutes, and progressed in severity over the course of several hours. These effects require direct dermal contact because similar effects on the skin are not observed following inhalation or oral exposure.

Ocular Effects. Double-vision was reported in an individual exposed dermally and by inhalatioin from weekly use of carbon tetrachloride fire-extinguishing fluid as a dry-cleaning agent over a period of 3 months (Forbes 1944).

No data were located for ocular effects in animals exposed dermally.

3.2.3.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans or animals after dermal exposure to carbon tetrachloride

3.2.3.4 Neurological Effects

A case of polyneuritis was reported in a man who had repeated dermal contact 8 hours/day with carbon tetrachloride using it as a degreasing agent (Farrell and Senseman 1944). Sudden severe epileptiform seizure and coma occurred in a subsequently fatal case following weekly combined inhalation/dermal exposure (Forbes 1944). The individual, an alcoholic, was one of three men who misused a carbon tetrachloride fire-extinguishing fluid as a dry-cleaning agent over a period of 3 months (Forbes 1944).

No studies were located regarding neurological effects in animals after dermal exposure to carbon tetrachloride.

3.2.3.5 Reproductive Effects

No studies were located regarding reproductive effects in humans or animals after dermal exposure to carbon tetrachloride.

3.2.3.6 Developmental Effects

No studies were located exclusively regarding developmental effects in humans or animals after dermal exposure to carbon tetrachloride. However, note the epidemiological studies discussed in Section 3.2.2.6,

which almost certainly involved significant dermal and inhalation exposures in addition to the emphasized oral exposure.

3.2.3.7 Cancer

No studies were located regarding carcinogenic effects in humans or animals following dermal exposure to carbon tetrachloride.

3.3 GENOTOXICITY

The genotoxic potential of carbon tetrachloride has been evaluated *in vivo* (Table 3-5) and *in vitro* (Table 3-6).

Inhalation Exposure. No studies were located on genetic effects in humans or animals after inhalation exposure to carbon tetrachloride.

Oral Exposure. No studies were located regarding genetic effects in humans after oral exposure to carbon tetrachloride.

Results of *in vivo* tests in animals orally exposed to carbon tetrachloride suggest that genotoxic effects only occur at doses high enough to cause hepatic toxicity. No sex-linked recessive mutations were induced in *Drosophila melanogaster* exposed dietarily (Foureman et al. 1994). In oral gavage studies, there were no increases in the frequencies of chromosomal aberration, sister chromatid exchange, or micronucleus formation in the liver of rats or in the frequency of micronucleus formation in bone marrow of mice (Sawada et al. 1991; Suzuki et al. 1997). Some studies reported negative results in the liver for unscheduled DNA synthesis in rats (Mirsalis and Butterworth 1980; Mirsalis et al. 1982). However, authors of one study attributed increased DNA synthesis in rats exposed at a high dose not to unscheduled DNA synthesis *per se*, but to the increased tissue regeneration following hepatic necrosis (Craddock and

Table 3-5. Genotoxicity of Carbon Tetrachloride *In Vivo*

Species (test system)	End point	Results	Reference
Oral route:			
Drosophila melanogaster[a]	Sex-linked recessive mutation	–	Foureman et al. 1994
Rat hepatocytes[b]	Chromosomal aberrations	–	Sawada et al. 1991
Rat hepatocytes[b]	Sister chromatid exchange	–	Sawada et al. 1991
Rat hepatocytes[b]	Micronuclei	–	Sawada et al. 1991
Rat hepatocytes[c]	Unscheduled DNA synthesis	–	Mirsalis and Butterworth 1980
Rat hepatocytes[d]	Unscheduled DNA synthesis	–	Mirsalis et al. 1982
Rat hepatocytes[e]	Unscheduled DNA synthesis	–	Craddock and Henderson 1978
Rat hepatocytes[f]	Unscheduled DNA synthesis	[+]	Craddock and Henderson 1978
Rat hepatocytes[g]	DNA damage	–	Bermudez et al. 1982
Rat liver[h]	DNA adducts (lipid peroxidation)	+	Chaudhary et al. 1994
Rat liver (partially hepatectomized)[i]	Caffeine elutable (single-stranded) DNA	–	Stewart 1981
Hamster liver and kidney[j]	DNA adducts (lipid peroxidation)	+	Wang and Liehr 1995
Mouse liver, stomach, kidney, bladder, lung, brain, bone marrow[k]	DNA damage (comet assay) after 3 hours	–	Sasaki et al. 1998
Mouse stomach, kidney, bladder, lung, brain, bone marrow[l]	DNA damage (comet assay) after 24 hours	–	Sasaki et al. 1998
Mouse liver[m]	DNA damage (comet assay) after 24 hours	[+]	Sasaki et al. 1998
Mouse liver[n]	DNA damage (alkaline elution) after 4 hours	–	Schwartz et al. 1979
Mouse bone marrow[o]	Micronuclei after 24–72 hours	–	Suzuki et al. 1997
Intraperitoneal injection:			
D. melanogaster[p]	Sex-linked recessive mutation	–	Foureman et al. 1994
Rat forestomach, liver, lung, colon, kidney[q]	DNA adducts (lipid peroxidation)	[+]	Wacker et al. 2001
Rat liver[r]	DNA adducts (lipid peroxidation)	[+]	Chung et al. 2001
Rat liver[s]	Covalent binding to DNA	+	Castro et al. 1989
Hamster liver[s]	Covalent binding to DNA	+	Castro et al. 1989
Mouse liver[s]	Covalent binding to DNA	+	Castro et al. 1989
Mouse peripheral reticulocytes[t]	Micronuclei	–	Suzuki et al. 1997
Mouse bone marrow[u]	Micronuclei after 24 or 48 hours	–	Crebelli et al. 1999

Table 3-5. Genotoxicity of Carbon Tetrachloride *In Vivo*

Species (test system)	End point	Results	Reference
Oral route:			
Rat liver[v]	DNA damage (reduced viscosity) after 2 hours	–	Brambilla et al. 1983
Rat liver[w]	DNA damage (alkaline elution) after 4 hours	–	Barbin et al. 1983

[a]Males exposed to dietary concentration of 25,000 ppm for 72 hours prior to mating with untreated females.
[b]Male F344 rats exposed to 1,600 mg/kg carbon tetrachloride by oral gavage in corn oil 4–72 hours prior to sacrifice.
[c]Male F344 rats exposed to 100 mg/kg carbon tetrachloride by oral gavage in corn oil.
[d]Male F344 rats exposed to 400 mg/kg carbon tetrachloride by oral gavage in corn oil.
[e]Female Wistar rats exposed to 4,000 mg/kg carbon tetrachloride by oral gavage in liquid paraffin; injected with hydroxyurea to stop *de novo* DNA synthesis and tritiated thymidine two hours after dosing.
[f]Female Wistar rats exposed to 4,000 mg/kg carbon tetrachloride by oral gavage in liquid paraffin; injected with hydroxyurea to stop *de novo* DNA synthesis and tritiated thymidine 17 hours after dosing. Increase in DNA synthesis associated with increased tissue regeneration, but no unscheduled DNA synthesis.
[g]Male Fischer 344 rats exposed to 400 mg/kg carbon tetrachloride by oral gavage in corn oil; nuclei of hepatocytes isolated 2, 12, or 24 hours after dosing were analyzed by alkaline elution.
[h]Sprague-Dawley rats (sex not specified) exposed to 0.1 mg/kg carbon tetrachloride by oral gavage 4 days before sacrifice.
[i]Female Wistar rats, 3 weeks after partial hepatectomy and treatment with tritiated thymidine, exposed to 200–800 mg/kg carbon tetrachloride by oral gavage in corn oil 4 or 24 hours before sacrifice.
[j]Female Syrian golden hamsters exposed to 160 or 1,600 mg/kg carbon tetrachloride by oral gavage in corn oil 4 days prior to sacrifice.
[k]Male CD-1 mice, exposed to 2,000 mg/kg carbon tetrachloride by oral gavage in corn oil.
[l]Male CD-1 mice, exposed to 500, 1,000, or 2,000 mg/kg carbon tetrachloride by oral gavage in corn oil.
[m]Male CD-1 mice, exposed to hepatotoxic doses of 1,000 or 2,000 mg/kg carbon tetrachloride by oral gavage in corn oil; no effect observed at 500 mg/kg.
[n]Male and female NMRI mice exposed to 4,000 mg/kg carbon tetrachloride by oral gavage in corn oil 4 hours before sacrifice.
[o]Male BDF_1 mice exposed to 500, 1,000, or 2,000 mg/kg carbon tetrachloride by oral gavage in olive oil 24 hours before sacrifice.
[p]Males injected with 0.7% NaCl containing 2,000 ppm carbon tetrachloride in ethanol 24 hours before mating.
[q]Female F344 rats injected with 500 mg/kg carbon tetrachloride 4–24 hours before sacrifice.
[r]Male F344 rats injected with 3,200 mg/kg carbon tetrachloride in olive oil.
[s]Animals injected with 770 mg/kg radiolabeled carbon tetrachloride 6 hours before sacrifice.
[t]Male BDF_1 mice injected with 2,000 or 3,000 mg/kg carbon tetrachloride 24–72 hours before sacrifice.
[u]Male or female CD-1 mice injected with 1,500 or 3,000 mg/kg carbon tetrachloride 24–48 hours before sacrifice.
[v]Male Sprague-Dawley rats injected with 200 mg/kg carbon tetrachloride.
[w]Male BD-V1 rats injected with 4,000 mg/kg carbon tetrachloride.

– = negative result; + = positive result; [+] = hepatotoxicity was evident; DNA = deoxyribonucleic acid

Table 3-6. Genotoxicity of Carbon Tetrachloride *In Vitro*

Test system Species (concentration)[a]	End point	Results: With activation	Results: Without activation	Reference
Prokaryotic organisms:				
Escherichia coli K-12 343/113 (15.4 mg/mL)	Differential DNA repair	–	–	Hellmer and Bolcsfoldi 1992
E. coli WP2, WP67, CM871; liquid micromethod, sealed (12.5 µg)	Differential DNA repair	+	+	De Flora et al. 1984
E. coli WP2, WP67, CM871; 2-hour preincubation, sealed	Differential DNA repair	–	+	De Flora et al. 1984
E. coli WP2, WP67, CM871; spot test	Differential DNA repair	NT	–	De Flora et al. 1984
E. coli PQ37 (1540 µg/mL)	SOS induction (DNA repair)	–	–	Brams et al. 1987
E. coli WP2*/pKM101; WPuvrA/pKM101* (1,000 ppm; enclosed gas atmosphere 24 hours)	Reversion frequency	+	+	Araki et al. 2004
E. coli WPuvrA/pKM101 (10,000 ppm; enclosed gas atmosphere 24 hours)	Reversion frequency	+/–	+/–	Araki et al. 2004
Salmonella typhimurium TA98 (10,000 ppm; enclosed gas atmosphere 24 hours)	Reversion frequency	–	+/–	Araki et al. 2004
S. typhimurium TA100, TA1535, TA1537 (50,000 ppm)	Reversion frequency	–	–	Araki et al. 2004
S. typhimurium TA100, TA1535 (10 mg/plate)	Reversion frequency	–	–	McCann et al. 1975
S. typhimurium TA1535, TA1538 (1,230 µg/mL)	Reversion frequency	–	No data	Uehleke et al. 1977
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	Reversion frequency	No data	–	Simmon et al. 1977
S. typhimurium TA1535, TA98, TA100) (2,830 µg/plate)	Reversion frequency	–	–	Barber et al. 1981
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538 (10 mg/plate)	Reversion frequency	–	–	De Flora 1981, De Flora et al.1984
S. typhimurium TA1537, TA100 (2,450 µg/plate)	Reversion frequency	+[b]	+[b]	Varma et al. 1988
S. typhimurium TA98 (2,450 µg/plate)	Reversion frequency	NT	+[b]	Varma et al. 1988
S. typhimurium TA1535 (2,450 µg/plate)	Reversion frequency	NT	–	Varma et al.1988
S. typhimurium TA1535/ pSK1002 (5.3 mg/plate)	SOS induction (*umu* expression)	–	–	Nakamura et al. 1987

Table 3-6. Genotoxicity of Carbon Tetrachloride *In Vitro*

Test system Species (concentration)[a]	End point	Results: With activation	Results: Without activation	Reference
S. typhimurium BA13 and BAL13; sealed (1,230 µg/plate)	Forward mutation (Ara^R test)	–	–	Roldan-Arjona et al. 1991
Eukaryotic organisms:				
Aspergillus nidulans P1 (diploid) (0.5%)	Somatic segregation, crossovers, non-disjunction frequency	NT	+ (CT)	Gualandi 1984
A. nidulans P1 (diploid) (0.0275%)	Somatic segregation	NT	+ (CT)	Benigni et al. 1993
A. nidulans 35 (haploid); (growth mediated) (0.5%)	Forward mutation (su meth G1)	NT	+ (CT)	Gualandi 1984
A. nidulans 35 (haploid); plate incorporation (0.5%)	Forward mutation (su meth G1)	NT	–	Gualandi 1984
Saccharomyces cerevisiae D7 (5230 µg/mL)	Frequency of convert-ants recombinants, revertants	No data	+	Callen et al. 1980
S. cerevisiae RS112 (diploid) (4 mg/mL)	DEL (intrachromosomal recombinant HIS^+)	NT	+ (CT)	Schiestl et al. 1989; Brennan and Schiestl 1998
S. cerevisiae RS112 (diploid) (4 mg/mL)	Interchromosomal recombinant (ADE^+)	NT	–	Schiestl et al. 1989
S. cerevisiae RS112 (diploid) (4 mg/mL)	DEL (intrachromosomal recombinant HIS^+)	NT	+/– (see text)	Galli and Schiestl 1998
S. cerevisiae RS112 (diploid); arrested in G1 (5 mg/mL)	Interchromosomal recombinant (ADE^+)	NT	+ (CT)	Galli and Schiestl 1996
S. cerevisiae RS112 (diploid); arrested in G1 (8 mg/mL)	DEL (intrachromosomal recombinant HIS^+)	NT	+ (CT)	Galli and Schiestl 1996
S. cerevisiae AGY3 (arrested in G2) (8 mg/mL)	DEL (intrachromosomal recombinant HIS^+)	NT	+ (CT)	Galli and Schiestl 1995
S. cerevisiae D61.M (6.4 mg/mL)	Aneuploidy	NT	–	Whittaker et al. 1989
Mammalian cells:				
Rat liver cell line (RL_1) (0.02 µg/mL)	Chromatid gaps, deletions or aberrations	No data	–	Dean and Hodson-Walker 1979
Rat hepatocytes (Wistar) (154 µg/mL)	DNA strand breaks, adducts	NT	+	Beddowes et al. 2003
Rat hepatocytes (460 µg/mL)	DNA damage	NT	+(CT)	Sina et al. 1983
Rat hepatocytes (CD) (154 µg/mL)	Unscheduled DNA synthesis	NT	–	Selden et al. 1994
Human/peripheral lymphocytes (48 µg/mL)	Sister chromatid exchange	–	–	Garry et al. 1990
Human/peripheral lymphocytes (76 µg/mL)	Chromosomal aberration	–	–	Garry et al. 1990
Human/ peripheral lymphocytes (1,540 µg/mL)	Micronuclei	+[c]	+[c]	Tafazoli et al. 1998

Table 3-6. Genotoxicity of Carbon Tetrachloride *In Vitro*

Test system Species (concentration)[a]	End point	Results With activation	Results Without activation	Reference
Human/ peripheral lymphocytes (3,080 µg/mL)	DNA damage (comet assay)	–	–	Tafazoli et al. 1998
Human/peripheral lymphocytes (16 mg/mL)	Unscheduled DNA synthesis	+/– (CT)	–	Perocco and Prodi 1981
Human lymphoblastoid cells AHH-1 (1,540 µg/mL)	Micronucleus formation	NT	–	Doherty et al. 1996
Human lymphoblastoid cells h2E1, MCL-5 (308 µg/mL)	Micronucleus formation	NT	+	Doherty et al. 1996
Lamb *(Ovis aries)*/peripheral lymphocytes (16 µg/mL)	Chromosomal aberration	NT	–	Sivikova et al. 2001
Lamb *(Ovis aries)*/peripheral lymphocytes (4 µg/mL)	Micronucleus formation	+	+	Sivikova et al. 2001
Chinese hamster ovary cells (1,490 µg/mL)	Sister chromatid exchange	–	–	Loveday et al. 1990
Chinese hamster ovary cells (3,000 µg/mL)	Chromosomal aberration	–	–	Loveday et al. 1990
Chinese hamster ovary cells (8 mg/mL)	Chromosomal aberration at anaphase	NT	+	Coutino 1979
Chinese hamster V79 cells (246 µg/mL)	Aneuploidy	NT	+	Onfelt 1987
Chinese hamster V79 cells	Spindle disturbances (c-mitosis)	NT	+ (CT)	Onfelt 1987
Syrian hamster embryo cells (3 µg/mL)	Clonal transformation	NT	+/-	Amacher and Zelljadt 1983
Calf thymus DNA (308 µg/mL)	Covalent binding	+	NT	DiRenzo et al. 1982

[a]Concentrations are the highest tested in negative studies and the lowest tested in negative studies.
[b]Effect not dose-related but cytotoxicity not evaluated.
[c]Effect not dose-related, seen only in cells from one of two subjects.

– = negative result; + = positive result; +/– = weak positive; CT = increase with cytotoxicity; NT = not tested; plate = plate incorporation assay; sealed = assay vessel sealed to prevent evaporation

Henderson 1978). Single high doses of carbon tetrachloride resulted in DNA breakage in the liver (but not other tissues) that was detectable electrophoretically (comet assay) in mice, but only 24 hours after dosing (Sasaki et al. 1998). No increase in DNA breakage was detected in mice assayed only 3 or 4 hours after receiving a high dose (Sasaki et al. 1998; Schwarz et al. 1979) or in rats exposed at a lower dose (Bermudez et al. 1982). Stewart (1981) found no increase in the generation of DNA repair intermediates (with accessible single-strand regions) in the livers of partially hepatectomized female Wistar rats given doses insufficient to cause overt hepatic necrosis 24 hours after exposure. The results of these studies suggest that DNA breakage resulting from oral exposure to carbon tetrachloride is related to hepatic cytotoxicity.

Some genotoxicity of carbon tetrachloride following oral exposure is related to the lipid peroxidation activity of its metabolites. Increases in endogenous lipid peroxidation adducts of DNA, such as malondialdehyde deoxyguanosine, were noted in the liver and kidney of hamsters and the liver of rats dosed with carbon tetrachloride (Chaudhary et al. 1994; Wang and Liehr 1995).

Dermal Exposure. No studies were located regarding genotoxic effects in humans or animals after dermal exposure to carbon tetrachloride.

Other Routes of Exposure In Vivo. Results of genotoxicity assays in which carbon tetrachloride was administered by intraperitoneal injection were similar to results from oral gavage assays. Carbon tetrachloride caused no increase in frequencies of sex-linked recessive mutations in *D. melanogaster* (Foureman et al. 1994) or micronucleus formation in mice (Crebelli et al. 1999; Suzuki et al. 1997). Hepatic DNA damage was not detectable electrophoretically in rats assayed 2–4 hours after exposure (Barbin et al. 1983; Brambilla et al. 1983).

Two kinds of DNA adducts related to carbon tetrachloride metabolism have been detected in injection studies. Covalent binding of metabolites of radiolabeled carbon tetrachloride was detected in the hepatic DNA of rats, hamsters, and mice 6 hours after injection (Castro et al. 1989); the level of binding to DNA was similar in the three species, whereas binding to nuclear proteins was 3 times higher in mice and hamsters than in rats. In other rat studies, carbon tetrachloride significantly increased the level of an endogenous lipid peroxidation product, *trans*-4-hydroxy-2-nonenal, and its deoxyguanosine adduct, 1,N^2-propanodeoxyguanosine (Chung et al. 2000; Wacker et al. 2001). The affected tissues included the liver and forestomach, and to a lesser degree the lung, colon, and kidney (Chung et al. 2000; Wacker et al. 2001). Hepatic increases in adduct formation were about 2-fold following a dose of 500 mg/kg and ~37-

fold following a dose of 3,200 mg/kg (Chung et al. 2000). *In vivo* genotoxicity studies are summarized in Table 3-5.

In Vitro. The genotoxicity of carbon tetrachloride has been studied in prokaryotic and eukaryotic cells *in vitro* (Table 3-6).

The majority of mutagenicity assays for bacteria exposed to carbon tetrachloride have been negative with or without metabolic activation, but volatilization of the chemical in standard plate incorporation methods using unsealed plates may have contributed to some negative findings. In *Salmonella typhimurium*, negative results were reported for a preincubation forward mutation assay in strains BA13 and BAL13 using sealed plates (Roldan-Arioina et al. 1991), for an SOS induction assay in strain TA1535/pSK1002 (Nakamura et al. 1987), and for reverse mutation assays in several strains (Araki et al. 2004; Barber et al. 1981; De Flora 1981, De Flora et al. 1984; McCann et al. 1975; Simmon et al. 1977; Uehleke et al. 1977; Varma et al. 1988). Increases in reverse mutation frequency were observed in plate incorporation assays for strains TA1537 and TA100 with or without activation and TA98 without activation, but the responses were not dose-related and cytotoxicity was not examined (Varma et al. 1988). Weak positive results for mutagenicity were also reported for strain TA98 exposed to 10,000 ppm carbon tetrachloride vapor in an enclosed system (Araki et al. 2004). In the same gas-phase system, weakly increased reversion frequencies with or without metabolic activation were reported for *Escherichia coli* strain WPuvrA/pKM101 and stronger increases for strain WP2/pKM101, which is repair-proficient. Negative results were reported for an SOS induction assay in *E. coli* strain PQ37 (Brams et al. 1987) and a differential DNA repair assays in strains K-12 343/113 (Hellmer and Bolcsfoldi 1992). Induction of differential DNA repair was observed in strains WP2, WP67, and CM871 when assays were conducted in sealed vessels, but not when conducted as spot tests (De Flora et al. 1984).

Assays for the genotoxicity (somatic segregation, non-disjunction frequency, forward mutation) of carbon tetrachloride in the mold *Aspergillus nidulans* were negative or positive only with cytotoxicity (Benigni et al. 1993; Gualandi 1984). Carbon tetrachloride did not induce aneuploidy in the yeast *Saccharomyces cerevisiae* strain D61.M (Whittaker et al. 1989). Similarly, cytotoxicity was generally observed at concentrations at which positive or weak positive results were reported for genotoxicity (interchromosomal recombination or reversion) in *S. cerevisiae* (Brennan and Schiestl 1998; Callen et al. 1980; Galli and Schiestl 1996). Galli and Schiestl (1998) observed that carbon tetrachloride induced intrachromosomal recombination in dividing cells or cells arrested in G1 or G2 phase, but not cells in S-phase. These authors suggested that chemical-induced cytotoxicity prematurely pushed G1 cells into

S-phase, indicating that genotoxicity might result from the failure to completely repair DNA before replication, resulting in DNA strand breaks.

In vitro genotoxicity assays in mammalian cells treated with carbon tetrachloride gave results consistent with *in vivo* bioassays. Carbon tetrachloride yielded weak positive results (1 out of 2,003 clones counted) in a clonal transformation assay in hamster embryo cells exposed without activation (Amacher and Zelljadt 1983). No increase in unscheduled DNA synthesis was observed in rat hepatocytes or human peripheral lymphocytes treated without metabolic activation, although weak positive results were observed in human lymphocytes treated with activation at cytotoxic concentrations (Perocco and Prodi 1981; Selden et al. 1994). Negative results in standard chromosomal aberration assays were reported for exposed rat hepatocytes, human peripheral lymphocytes, lamb lymphocytes, and Chinese hamster ovary cells (Dean and Hodson Walker 1979; Garry et al. 1990; Loveday et al. 1990; Sivikova et al. 2001). However, one study that examined Chinese hamster ovary cells for chromosomal aberrations at anaphase rather than metaphase reported 6-fold increases in lag chromosomes (indicative of centromere or microtubule malfunction) and multipolar spindles (Coutino 1979). Aneuploidy was induced in treated Chinese hamster V9 lung cells, but a 10% increase in spindle aberrations (c-mitosis) only occurred at a concentration at which 50% cytotoxicity was observed (Onfelt 1987). The frequency of sister chromatid exchanges was not elevated in human peripheral lymphocytes or Chinese hamster ovary cells treated with or without metabolic activation (Garry et al. 1990; Loveday et al. 1990). Micronucleus formation was induced in treated peripheral lymphocytes taken from one of two human donors (Tafazoli et al. 1998). In micronucleus assays in cultured human lymphoblastoid cells, negative results were reported for a cell line (AHH-1) with a low native level of CYP1A1 activity, but positive results for derivative cell lines expressing cDNA for one or more human microsoal enzymes (CYP2E1 in line h2E1 and CYP 1A2, 2A6, 3A4, and 2E1 and epoxide hydrolase in line MCL-5) (Doherty et al. 1996). These results demonstrate that biotransformation of carbon tetrachloride is needed for micronucleus induction. Increases in single-strand DNA breaks were observed in rat hepatocytes exposed to cytotoxic concentrations of carbon tetrachloride, but not in exposed human lymphocytes (Beddowes et al. 2003; Sina et al. 1983; Tafazoli et al. 1998)

As reported for *in vivo* studies, DNA adducts have been detected in mammalian cells following exposure to carbon tetrachloride *in vitro*. Covalent binding of radiolabeled carbon tetrachloride to DNA and protein was detected in hepatic nuclear preparations from male Sprague-Dawley rats, Syrian Golden hamsters, and C3H mice (Castro et al. 1989). When NADPH was added to the reaction mixtures, the level of covalent binding to DNA was enhanced for hamsters and mice, but not rats. Direct covalent

binding of carbon tetrachloride to calf thymus DNA was detected following microsomal bioactivation (DiRenzo et al. 1983); the degree of binding was increased 2.2-fold when the DNA samples were pre-treated with pronase, suggesting that reactive metabolites of carbon tetrachloride react with both DNA and protein. Carbon tetrachloride treatment of rat hepatocytes or hepatic microsomes also increased the frequency of DNA adducts generated as by-products of lipid peroxidation (Beddowes et al. 2003; Castro et al. 1997). In treated hepatocytes, there were statistically significant increases (compared to background levels) in malondialdehyde deoxyguanosine adducts (Beddowes et al. 2003). Increases in 8-oxodeoxy-guanosine adducts were observed at the threshold of, and concomitant with, cytotoxicity. A biochemical study using DNA bases and liver microsomes from male Sprague-Dawley rats demonstrated that the bioactivation of carbon tetrachloride resulted in the formation of adducts to guanine (2,6-diamino-4-hydroxy-5-formamidopyrimidine), cytosine (5-hydroxycytosine), and thymidine (5-hydroxy-methyluracil), but not to adenine (Castro et al. 1997). The authors attributed formation of these adducts to reactive metabolites (trichloromethyl or trichloromethylperoxyl free radicals) or to reactive aldehydes, such as malondialdehyde, which are generated by lipid peroxidation. *In vitro* genotoxicity studies are summarized in Table 3-6.

3.4 TOXICOKINETICS

Carbon tetrachloride is absorbed readily from the gastrointestinal and respiratory tracts, and more slowly through the skin. It is distributed to all major organs, with highest concentrations in the fat, liver, bone marrow, adrenals, blood, brain, spinal cord, and kidney (Bergman 1983; Dambrauskas and Cornish 1970; McCollister et al. 1951; Paustenbach et al. 1986a, 1986b). Once carbon tetrachloride is absorbed, it is metabolized by cytochrome P-450 enzymes, with the production of the trichloromethyl radical (Lai et al. 1979; Poyer et al. 1978). Aerobically, metabolism of the trichloromethyl radical can eventually form phosgene (Shah et al. 1979). Anaerobically, the radical can undergo reactions to form chloroform (Glende et al. 1976; Uehleke et al. 1973), hexachloroethane (Fowler 1969; Uehleke et al. 1973), or carbon monoxide (Wolf et al. 1977), as well as bind directly to lipids, proteins, and deoxyribonucleic acid (DNA) (Rao and Recknagel 1969). Carbon tetrachloride is excreted primarily in exhaled air (initial elimination half-life of 1–3 hours) and in the feces, while relatively minimal amounts are excreted in the urine (McCollister et al. 1951; Paustenbach et al. 1986a; Stewart and Dodd 1964; Stewart et al. 1961, 1963, 1965; Young and Mehendale 1989).

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

Although there are many cases of human overexposure to carbon tetrachloride vapor, there are few quantitative studies of pulmonary absorption of carbon tetrachloride in humans. Based on the difference in carbon tetrachloride concentration in inhaled and exhaled air, absorption across the lung was estimate to be about 60% in humans (Lehmann and Schmidt-Kehl 1936). Monkeys exposed to 50 ppm absorbed an average of 30.4% of the total amount of carbon tetrachloride inhaled, at an average absorption rate of 0.022 mg carbon tetrachloride/kg/minute (McCollister et al. 1951). The concentration of carbon tetrachloride in the blood increased steadily in monkeys, but did not reach a steady-state within 344 minutes of exposure. In rats exposed to 100 or 1,000 ppm for 2 hours, the total absorbed dose of carbon tetrachloride was 17.5 or 179 mg/kg of body weight, respectively (Sanzgiri et al. 1995). (These results were used to establish dose levels for parallel oral-route studies described in Section 3.4.1.2.) Carbon tetrachloride was rapidly absorbed from the lungs as indicated by the near peak levels that were measured in arterial blood at the earliest timepoint (5 minutes). A near steady-state was achieved within 10 or 15 minutes and was maintained for the duration of the 2-hour exposures. In rats, mice, and hamsters exposed to 20 ppm ^{14}C-labeled carbon tetrachloride vapor for 4 hours, the initial body burdens of carbon tetrachloride equivalents (CE) immediately following exposure were 12.1, 1.97, and 3.65 μmol, respectively (Benson et al. 2001).

3.4.1.2 Oral Exposure

No studies were located regarding absorption in humans after oral exposure to carbon tetrachloride. It would be anticipated, however, that carbon tetrachloride is well absorbed from the gastrointestinal tract of humans, since carbon tetrachloride is readily absorbed from the gastrointestinal tract of animals (see below), and there are many accounts of human poisonings resulting from ingestion of carbon tetrachloride (e.g., Ashe and Sailer 1942; Conway and Hoven 1946; Gosselin et al. 1976; Guild et al. 1958; Kluwe 1981; Lamson et al. 1928; Phelps and Hu 1924; Ruprah et al. 1985; Stewart et al. 1963; Umiker and Pearce 1953; von Oettingen 1964).

Results from several animal studies indicate that carbon tetrachloride is rapidly and extensively absorbed from the gastrointestinal tract. Typically, 80–85% of an oral dose may be recovered in expired air, indicating that gastrointestinal absorption is at least 85% (Marchand et al. 1970; Paul and Rubinstein

1963). The time course of absorption depends on exposure conditions, with peak blood levels occurring as early as 3–6 minutes after dosing (Kim et al. 1990a). While oral absorption from water or other aqueous vehicles is very rapid and extensive, when carbon tetrachloride is administered using corn oil as the vehicle, absorption is slowed and diminished (Gillespie et al. 1990; Kim et al. 1990a). Similar findings were reported by Withey et al. (1983) for several other halogenated hydrocarbons. The absorption rate and, therefore, peak blood levels will be inversely proportional to the volume of corn oil employed in oral dosing.

Sanzgiri et al. (1995) compared pharmacokinetics of carbon tetrachloride administered to fasted rats as a single bolus by gavage or by infusion over 2 hours. The doses, 17.5 and 179 mg/kg, were established by uptake measured in a 2-hour inhalation experiment (see Section 3.4.1.1). Carbon tetrachloride was rapidly absorbed in the gastrointestinal tract. Peak arterial blood concentrations were reached within 15 minutes of bolus administration and then declined, whereas infusion caused a steady increase over the 2-hour period. The peak concentrations were higher for the bolus group than for the infusion group.

3.4.1.3 Dermal Exposure

Carbon tetrachloride is significantly absorbed through the skin of humans, though less readily than from the lung or gastrointestinal tract. When volunteers immersed their thumbs in undiluted carbon tetrachloride for 30 minutes, carbon tetrachloride was detected in the alveolar air of each subject within 10 minutes, indicating relatively rapid percutaneous absorption (Stewart and Dodd 1964). The alveolar concentration of carbon tetrachloride rose steadily thereafter and peaked by about 30 minutes postexposure. The authors estimated that immersion of both hands in liquid carbon tetrachloride for 30 minutes would yield an exposure equivalent to breathing 100–500 ppm for 30 minutes. The investigators noted that the amount of carbon tetrachloride that can penetrate human skin appeared to be related to the method of application, the duration and area of skin exposure, and the type of skin exposed.

Studies in animals confirm that liquid carbon tetrachloride is absorbed through the skin (Jakobson et al. 1982; Morgan et al. 1991; Tsuruta 1975). The rate of uptake is high enough (54 nmol/min/cm^2 in mice) that absorbed doses may be comparable to the doses absorbed from relatively high levels of carbon tetrachloride in air (Tsuruta 1975). Uptake kinetics are linear only for a short time (about 30 minutes), after which blood levels tend to decrease (Jakobson et al. 1982; Morgan et al. 1991). This is probably due to local vasoconstriction in the exposed skin area. During the course of a 24-hour exposure (2 mL/3.1 cm^2 skin), rats absorbed 27% (0.54 mL) of the applied neat solution, whereas >99% of the

carbon tetrachloride in 110–648 μg/mL aqueous solutions (approximately one-third to completely saturated) was absorbed (Morgan et al. 1991). Rather broad peak blood concentrations of approximately 8–70 ng/mL were observed 2–8 hours into the exposure period. In monkeys, the dermal absorption of radioactive carbon tetrachloride vapor at concentrations of 485 or 1,150 ppm over a period of 240 or 270 minutes, respectively, was negligible, as measured in samples of blood and expired air (McCollister et al. 1951).

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

No studies were located regarding distribution in humans after inhalation exposure to carbon tetrachloride.

Inhalation studies in monkeys (McCollister et al. 1951), rats (Benson et al. 2001; Dambrauskas and Cornish 1970; Paustenbach et al. 1986a, 1986b; Sanzgiri et al. 1997), and hamsters and mice (Benson et al. 2001) reveal that the highest carbon tetrachloride concentrations occur in fat, and in organs or tissues with high fat content such as bone marrow, liver, brain, and kidney. In rats exposed to 1,000 ppm for 2 hours (receiving a dose of 179 mg/kg), the maximal concentration of carbon tetrachloride was reached within 30 minutes (the earliest timepoint) in the liver, kidney, lung, brain, heart, muscle, spleen, and gastrointestinal tract, and by 240 minutes in fat (Sanzgiri et al. 1997). The maximal concentration of carbon tetrachloride (μg/g tissue) achieved in the kidney was 1.25 times higher than the liver. The area under the tissue concentration versus time curve (AUC) for the first 30 minutes of exposure was 322, 409, 460, and 710 μg per minute/mL, respectively, for the liver, kidney, brain, and fat. The half-life of clearance from different organs (evaluated over 24 hours) ranged from 204 minutes for the kidney, 249 minutes for the liver to 665 minutes for fat. Through the use of a low temperature whole-body autoradiographic technique, Bergman (1983) observed a particularly high uptake of ^{14}C-carbon tetrachloride into the white matter of brain, spinal cord, and spinal nerves in mice exposed by inhalation. Considerably lower levels were found in the kidney, lung, spleen, muscle, and blood.

Immediately following exposure to 20 ppm ^{14}C-labeled carbon tetrachloride vapor for 4 hours, the proportion of the initial body burden as carbon tetrachloride equivalents (CE) present in the major tissues was 30% for rats and hamsters and 40% for mice (Benson et al. 2001). The CE concentrations at that

time were highest in the liver of mice and hamsters but were highest in fat for rats; 48 hours later, CE concentrations in all three species were highest in the liver.

3.4.2.2 Oral Exposure

No studies were located regarding distribution in humans after oral exposure to carbon tetrachloride.

Studies of the time-course of tissue distribution in male rats given oral doses of carbon tetrachloride reported that concentrations in the blood, striated muscle, brain, and liver were maximal 2 hours after dosing (Marchand et al. 1970). The peak concentrations in the liver and brain were significantly higher than in the muscle and blood. Peak levels in the fat were not reached until 5.5 hours post dosing, at which time they were more than 50-fold greater than peak blood levels. A similar time-course of tissue deposition of carbon tetrachloride has been observed in female rats (Teschke et al. 1983) and rabbits (Fowler 1969) dosed orally with carbon tetrachloride. Higher carbon tetrachloride levels were found consistently in the liver than in the brain of rats dosed orally (Marchand et al. 1970; Watanabe et al. 1986). This may be because carbon tetrachloride absorbed from the gastrointestinal tract enters the portal circulation, which initially passes through the liver. A significant proportion of the carbon tetrachloride is likely taken up from the portal blood during the first pass, resulting in the high liver levels following ingestion. One week after exposure to ^{14}C-carbon tetrachloride, the concentrations of radiolabel (expressed as mmol carbon tetrachloride/g tissue) were about 1.5 in plasma, 5–6.5 in soleus and white vastus lateralis muscle, 8 in liver, 10 in kidney and diaphragm, and 13 in adipose tissue (Weber et al. 1992). It is interesting to note that phenobarbital pretreatment, often used to hasten or intensify the toxic effects of carbon tetrachloride exposure, was found not only to nearly double the amount of radiolabel retained in the examined tissues, but also to significantly alter its distribution. Liver, kidney, and plasma concentrations were elevated to 600, 350, and 150% of their respective control (carbon tetrachloride alone) levels, while the muscle, diaphragm, and adipose levels were reduced to 40–70%. This observation is consistent with higher levels of the administered dose being metabolized (largely in the liver) and subsequently entering the carbon pool.

Sanzgiri et al. (1997) exposed rats by bolus dosing or gastric infusion over 2 hours to a dose of carbon tetrachloride that was equivalent to the amount absorbed during a 2-hour exposure at 1,000 ppm. In rats receiving a dose of 179 mg/kg by infusion over 2 hours, the maximal concentration of carbon tetrachloride was reached by 120 minutes in the liver, kidney, and heart, 150 minutes in the brain, muscle, and spleen, 180 minutes in lung, and by 360 minutes in fat (Sanzgiri et al. 1997). The AUC for the first

30 minutes of exposure was 3, 4, 28, and 157 µg per minute/mL, respectively, for the liver, kidney, brain, and fat in infused rats. Absorption of carbon tetrachloride was more rapid and organ concentrations of carbon were higher in rats that received the same dose as a single bolus by gavage. The maximal concentration was reached by 1 minute in the liver, 5 minutes in the kidney, heart, and spleen, 15 minutes in lung and brain, 60 minutes in muscle, and 120 minutes in fat. The AUC for the first 30 minutes was 680, 380, 423, and 306 µg per minute/mL, respectively, for the liver, kidney, brain, and fat in the bolus-treated rats. The authors indicated that the bolus-delivery resulted in high 30-minute AUC values because the capacity of first-pass hepatic and pulmonary elimination was exceeded. The half-life of clearance from different organs (based on the AUC over 24 hours) ranged from 190 minutes for the kidney and 269 minutes for the liver to 358 minutes for fat in the infused rats, and from 278 minutes for the kidney and 323 minutes for the liver to 780 minutes for fat in the bolus-treated rats. The maximum tissue concentrations (µg/g tissue) achieved in the kidney were 24% of the value for the liver following bolus dosing, but 8 times higher than the liver following gastric infusion.

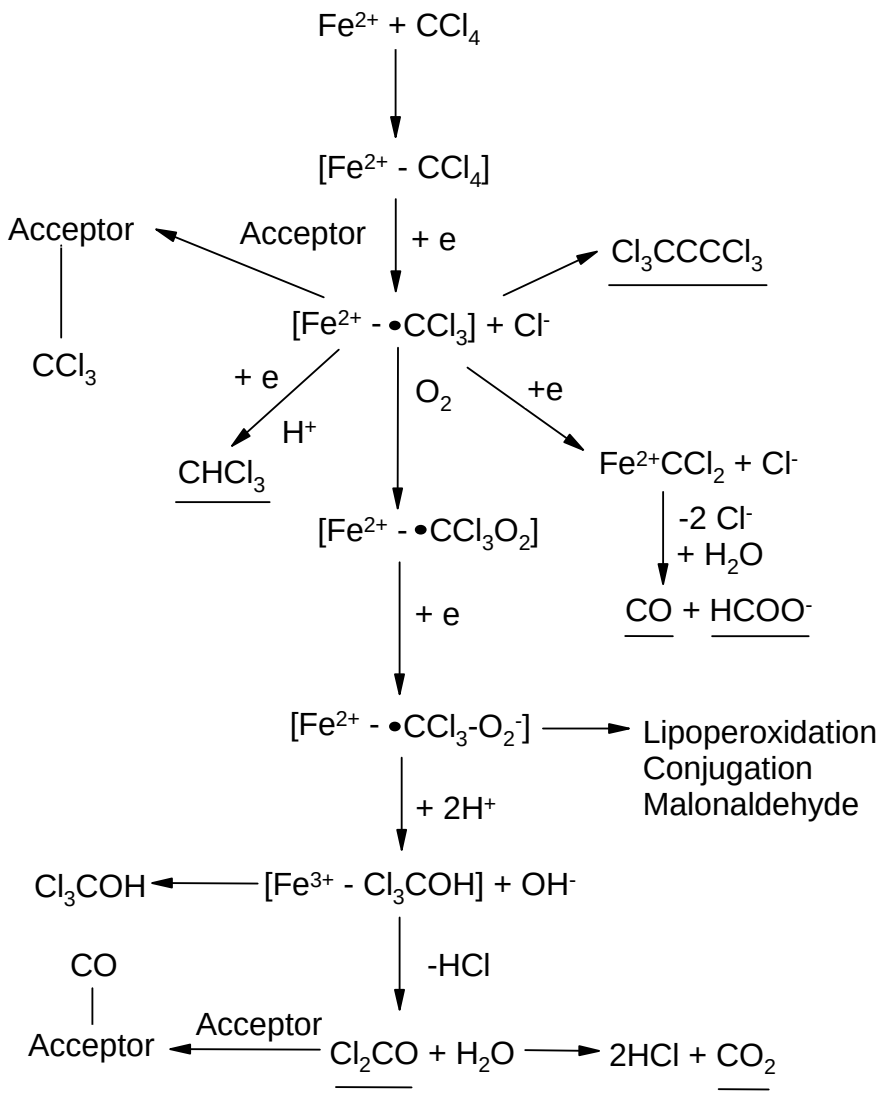
3.4.2.3 Dermal Exposure

No studies were located regarding distribution in humans or animals after dermal exposure to carbon tetrachloride.

3.4.3 Metabolism

The metabolism of carbon tetrachloride in humans has not been investigated, but a great deal of information is available from studies in animals. Pathways of carbon tetrachloride metabolism are illustrated in Figure 3-3, and metabolites that have been identified are underlined. Bioactivation of carbon tetrachloride proceeds by cytochrome P-450-dependent reductive dehalogenation (Sipes et al. 1977). Ethanol inducible CYP2E1 is the primary enzyme responsible for metabolizing carbon tetrachloride in humans at environmentally relevant concentrations, but others, particularly CYP3A, are also involved at higher concentrations (Castillo et al. 1992; Zangar et al. 2000). Studies with CYP2E1 genetic knockout mice *(cyp2e1$^{-/-}$)* demonstrated that hepatic toxicity of carbon tetrachloride in mice is entirely dependent on CYP2E1 (Wong et al. 1998). A large body of experimental data indicates that the first step involves homolytic cleavage of one carbon chlorine bond in carbon tetrachloride to yield chloride ion and the trichloromethyl radical (Lai et al. 1979; Poyer et al. 1978). Anerobically, the

Figure 3-3. Pathways of Carbon Tetrachloride Metabolism*



*Adapted from Shah et al. 1979. Fe2+ and Fe3+ denote the reduced and oxidized forms of cytochrome P-450, and brackets indicate an enzyme substrate complex. Electrons are donated from NADPH or NADH.

trichloromethyl radical may undergo several reactions, including (1) direct binding to microsomal lipids and proteins (Ansari et al. 1982; Gordis 1969; Rao and Recknagel 1969; Villarruel et al. 1977); (2) addition of a proton and an electron to form chloroform (Glende et al. 1976; Uehleke et al. 1973); (3) dimerization to form hexachloroethane (Fowler 1969; Uehleke et al. 1973); and (4) further reductive dechlorination to form carbon monoxide (Wolf et al. 1977). Aerobically, trichloromethyl radical (CCl_3•) may be trapped by oxygen to form trichloromethylperoxy radical (CCl_3OO•), which decomposes to phosgene ($COCl_2$) (Pohl et al. 1984). Hydrolytic cleavage of phosgene is likely the major pathway by which carbon dioxide is formed from carbon tetrachloride (Shah et al. 1979). The trichloromethylperoxy radical is more reactive than the trichloromethyl radical towards amino acids (Packer et al. 1978).

Metabolism of carbon tetrachloride by CYP2E1 may result in the destruction of the enzyme during the metabolic process (Noguchi et al. 1982a, 1982b) CYP2E1 may be lost by either a direct attack (i.e., covalent binding) of radicals on the cytochrome(s) (Manno et al. 1992; Vittozzi and Nastainczyk 1987), or highly localized lipid peroxidation resulting in detachment of P-450 proteins from the microsomal membranes. Cytochrome P-450 mediated homolytic cleavage of the carbon-chlorine bond in carbon tetrachloride is thought to be followed by hydrogen abstraction by the trichloromethyl radical at a methylene group of polyenic fatty acids in the microsomal lipids, thus forming organic free radicals. These organic free radicals then rapidly react with molecular oxygen, leading to the formation of organic peroxy free radicals and eventually organic peroxides (Rao and Recknagel 1969; Recknagel 1967; Recknagel and Glende 1973; Recknagel et al. 1977). The unstable organic peroxides cleave homolytically to form new free radicals, which attack methylene groups of neighboring polyenic lipids in the membrane. This autocatalytic process occurs very rapidly; hepatic microsomal lipid peroxidation is more than half of its maximum value at 5 minutes, and is complete within 15 minutes after oral administration of carbon tetrachloride to fasted rats (Rao and Recknagel 1968). Lipid peroxidation can contribute to breakdown of membrane structure and loss of organelle and cell functions. Connor et al. (1986) conducted a study in which they detected the trichloromethyl radical and a second free radical, the carbon dioxide anion radical, by electron spin resonance spectroscopy in liver perfusate and in urine of female rats. Adducts of both radicals have also been detected in blood of male rats (Reinke and Janzen 1991).

Cytochrome P-450 from rat or human liver microsome preparations is inactivated when incubated anaerobically with carbon tetrachloride in the presence of NADPH and an oxygen-scavenging system (Manno et al. 1988, 1992). Inactivation involved destruction of the heme tetrapyrrolic structure, and followed pseudo first-order kinetics with fast and slow half-lives of 4.0 and 29.8 minutes. When

compared with rat liver microsomes, the human preparations were 6–7 times faster at metabolizing carbon tetrachloride and only about one-eighth as susceptible to self-destructing ("suicidal") inactivation (about 1 enzyme molecule lost for every 196 carbon tetrachloride molecules metabolized).

The rate of carbon tetrachloride metabolism *in vivo* has been estimated primarily by indirect methods. Male rats were exposed to carbon tetrachloride vapor in a desiccator jar with a recirculating atmosphere. The decline in the chamber concentration was monitored over time as the index of carbon tetrachloride uptake into the animals (Gargas et al. 1986). The shapes of the uptake curves were a function of tissue partition coefficients and the metabolism of carbon tetrachloride. The uptake kinetics of carbon tetrachloride were accurately described by a physiological pharmacokinetic model with a single, saturable metabolic pathway. The maximum rate of reaction (Vmax) was calculated to be 0.14 mg/hour (0.62 mg/kg/hour), while the half-maximum rate concentration of carbon tetrachloride (K_m, the Michaelis-Menten constant) was calculated to be 1.62 μM (0.25 mg/L). Carbon tetrachloride was metabolized more slowly than other halocarbons studied (methyl chloroform, 1,1-dichloroethylene, bromochloromethane). Another indirect method was evaluated for estimating the rate of carbon tetrachloride metabolism in male rats, based on arterial blood:inhaled air concentration ratios (Uemitsu 1986). Results of this study suggest that carbon tetrachloride metabolism was limited by the rate of blood perfusion of the liver at concentrations below 100 ppm, and was saturated at concentrations above 100 ppm. The estimated Vmax was 2.8 mg/kg/hour. The rate of metabolism gradually decreased during the exposure period, apparently the result of carbon tetrachloride-induced loss of cytochrome P-450.

Based on comparative PBPK modeling, which incorporated *in vivo* gas uptake data and *in vitro* data, Thrall et al. (2000) calculated that the rates of metabolism (V_{max}/K_m) by milligrams of liver protein differed across species, with hamster > mouse > rat > human. The human *in vivo* metabolic rates for carbon tetrachloride were estimated as 1.49 mg/hour/kg body weight (V_{max}) and 0.25 mg/L for K_m.

The extent of metabolism of ^{14}C-carbon tetrachloride in rats was assessed by measuring the amounts of unchanged carbon tetrachloride, carbon dioxide, and chloroform exhaled in the breath, ^{14}C-metabolite excreted in urine and feces, and ^{14}C-metabolite bound to liver macromolecules within a 24-hour period post oral dosing (Reynolds et al. 1984). The major metabolite in this study was carbon dioxide at all dose levels, ranging from 85% of total metabolites recovered at 15 mg/kg to 63% at 4,000 mg/kg. The modest 22% (from 85 down to 63%) reduction in carbon dioxide production when the dose was increased 28-fold (15 versus 4,000 mg/kg) suggests that excess amounts of P-450 are available in the liver for metabolism of carbon tetrachloride. Intermediate amounts of nonvolatile ^{14}C-labeled material were recovered from

the urine and feces, although none of the metabolites were identified by these investigators. About 2–4% of the label was found covalently bound to liver macromolecules. The relative amount of chloroform formed depended on dose, with chloroform being the least abundant metabolite formed at the lowest dose, but the second most abundant metabolite at the highest dose. As the dose of carbon tetrachloride increased, the fraction of the dose recovered decreased for each metabolite except chloroform. A major change in the overall extent of carbon tetrachloride metabolism occurred as the dose was increased from 15 to 46 mg/kg, the nature of which suggests that the oxidative metabolism of carbon tetrachloride was saturated and/or impaired by destruction of cytochrome P-450 in this dosage range. The fraction recovered in the expired air as unchanged carbon tetrachloride increased from 20 to 80% of the administered dose, and the peak carbon tetrachloride exhalation rate increased 40-fold. Thus, this study indicated that when oxidative metabolism of carbon tetrachloride was saturated or inhibited, more of the parent chemical was exhaled and increased amounts of chloroform were formed by a reductive pathway. Low levels of carbon tetrachloride metabolism to CO_2 were also indicated by other studies showing that 6 hours after intraperitoneal injection of 128–159 mg/kg carbon tetrachloride to rats or gerbils, <1% (approximately 0.2% for rats, and 0.7% for gerbils) of the dose had been expired as CO_2, while approximately 80–90% had been expired as unchanged carbon tetrachloride (Cai and Mehendale 1990; Mehendale and Klingensmith 1988; Young and Mehendale 1989).

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

Little quantitative information was located regarding the amount or fraction of absorbed carbon tetrachloride that is subsequently excreted in air, urine, or feces in humans exposed by inhalation. Studies of the rate of excretion of carbon tetrachloride in the expired air indicate that samples taken immediately after exposure may be contaminated with environmental carbon tetrachloride, but realistic values may be obtained by 15 minutes (Stewart et al. 1961). Among volunteers who breathed 10–49 ppm carbon tetrachloride for 1–3 hours, expired air concentrations in the range of 1–3 ppm were detectable within 15-25 minutes, steadily declining to about 0.3 ppm 5 hours after exposure (Stewart et al. 1961). No carbon tetrachloride was detected in blood or urine of the subjects where the limit of detection was 5 ppm. In subjects who inhaled 2.5 mg radiochlorinated carbon tetrachloride vapor in a single breath (subsequently held for 20 seconds to maximize absorption), 33% of the administered dose was exhaled within 1 hour (Morgan et al. 1970). The urinary excretion rate during the first hour was less than 0.01% of the administered dose per minute.

Studies in animals indicate about 30–40% of an inhaled dose of carbon tetrachloride is excreted in expired air and about 32–62% is excreted in feces (McCollister et al. 1951; Paustenbach et al. 1986a). Relatively low amounts are excreted in urine. Nearly all of the material in expired air is parent carbon tetrachloride, with only small amounts of carbon dioxide. The identity of the nonvolatile metabolites in feces and urine was not determined.

During the 48 hours following nose-only inhalation exposure to 20 ppm ^{14}C-labeled carbon tetrachloride vapor for 4 hours, rats, mice and hamsters eliminated 65–83% of the initial body burden of ^{14}C activity as CO_2 or volatile organic compounds in exhaled breath (Benson et al. 2001). Elimination in expired air was described as a single-order negative exponential function. Elimination half-times for carbon tetrachloride equivalents (CEs) in exhaled breath were 4.3, 0.8, and 3.6 hours for volatile organic compounds and 7.4, 8.8, and 5.3 hours for CO_2 for rats, mice, and hamsters, respectively. The fraction of the initial body burden of CEs eliminated in urine and feces combined was <10% in rats and >20% in mice and hamsters. Clearance of CEs from various tissues was characterized as being best described by single- or two-component negative exponential functions (Benson et al. 2001). Clearance of CEs from the blood was complete within 48 hours and was described by a single-component function for all three species. The half-life for clearance ($T_{1/2}$) from blood was shortest for rats (1.8 hours) and longest for hamsters (23 hours). Clearance of CEs from the lung was also described by a single-component function for all three species, but was only about 80% complete after 48 hours; the $T_{1/2}$ ranged from 7 hours for rats to 17 hours for mice. Clearance of CEs from the liver in hamsters was complete and best described by a single-component function; the $T_{1/2}$ was 33 hours. In rats and mice, clearance from the liver was best described by a two-component function; a large fraction was cleared with a $T_{1/2}$ of 3 hours and the remainder cleared with a $T_{1/2}$ of 35 hours. Clearance of CEs from the kidney in rats was complete and best described by a two-component function. In mice and hamsters, the $T_{1/2}$ for clearance from the kidney for the largest fraction (70–80%) of carbon tetrachloride was <10 hours, but no additional clearance occurred up to 48 hours.

As in humans, the rate of carbon tetrachloride excretion in rats appears to be biphasic, with an initial half-life value of 7–10 hours (Paustenbach et al. 1986a). The rapid phase was judged to reflect clearance from blood, while the slower phase was related to clearance from fatty tissue and metabolic turnover of covalent adducts (Paustenbach et al. 1988). In support of this, exposure for longer periods of time led to higher concentrations of carbon tetrachloride in fat and a decreased rate of clearance (Paustenbach et al. 1986a, 1986b, 1988).

3.4.4.2 Oral Exposure

The concentration of carbon tetrachloride was measured in the expired air of a person who swallowed a large amount of carbon tetrachloride (Stewart et al. 1963). Excretion in expired air was found to decrease exponentially in a biphasic or multiphasic fashion, but no quantitative estimate of the elimination half-life of carbon tetrachloride or of the fraction of the dose excreted by this pathway was provided. Visual inspection of their graphed data suggests very approximate half-lives of less than several hours initially, 40 hours (75–150 hours post exposure), and 85 hours (300–400 hours post exposure).

A detailed investigation of carbon tetrachloride excretion was performed in rats exposed by gavage to a range of doses (Reynolds et al. 1984). At doses of 50 mg/kg or higher, most of the dose (70–90%) was recovered in expired air as unchanged carbon tetrachloride. Lower amounts were recovered as expired carbon dioxide or chloroform, or as nonvolatile metabolites in feces or urine. As would be expected for a saturable or self-destructing metabolic system, the proportion of each dose recovered as metabolites tended to decrease as the dose increased. For example, 12% of the lowest dose (15 mg/kg) was recovered as carbon dioxide, while only 0.7% of the highest dose (4,000 mg/kg) was recovered as carbon dioxide. The time-course of excretion also depended on dose, tending to become slower as doses increased. For example, the half-life for exhalation of carbon tetrachloride was 1.3 hours at a dose of 50 mg/kg, but was 6.3 hours at a dose of 4,000 mg/kg. This is consistent with the concept that an increased proportion of a dose enters fat as the dose level increases, with clearance from fat being slower than from blood and other tissues. Increased hepatotoxicity in the form of greater cytochrome P-450 destruction (and thus reduced carbon tetrachloride metabolism) may also be a significant factor. Studies evaluating the rate of excretion over the first 12 hours described a one-compartment model, but did not deduce that a two-compartment model was inappropriate (Reynolds et al. 1984). Approximately 24 hours after receiving an oral dose of 3,985 mg/kg, rats were observed to excrete elevated levels of various lipid peroxidation products (formaldehyde, acetaldehyde, malondialdehyde, and acetone) in their urine, presumably as a result of carbon tetrachloride-induced oxidative stress (Shara et al. 1992).

3.4.4.3 Dermal Exposure

Carbon tetrachloride was rapidly excreted in expired air of volunteers who immersed their thumbs in liquid carbon tetrachloride (Stewart and Dodd 1964). The half-life of expiration was about 30 minutes,

but no quantitative estimate of the fraction of the absorbed dose excreted in air was performed. No studies were located regarding excretion in animals after dermal exposure to carbon tetrachloride.

3.4.4.4 Other Routes of Exposure

After what was described as either intragastric or intraduodenal administration of carbon tetrachloride to rats under various conditions, evidence from electron paramagnetic resonance experiments using phenyl-N-t-butyl nitrone as a spin trap suggested that trichloromethyl free-radical adducts are secreted into the bile without being concentrated, and in concentrations which reflect those concurrently found in the liver (Knecht and Mason 1991). Expressed in arbitrary concentration units, spin-trap-bound adduct quantities found in the liver, in the bile, and liver/bile concentration ratios under the various experimental conditions were as follows: carbon tetrachloride alone (93, 28, 3.4 ratio), carbon tetrachloride plus hypoxia (161, 50, 3.2 ratio), carbon tetrachloride with phenobarbital pretreatment (118, 69, 1.7 ratio), and carbon tetrachloride with intravascular infusion of the bile salt dehydrocholate to double the bile flow rate (85, 13, 6.8 ratio). Taken together, these results from conditions that vary bile flow or reductive metabolic generation of free radical seem to indicate that carbon tetrachloride free-radical adducts are secreted rather than merely diffused into bile, and in amounts proportional to their generation in the liver. The drop in liver/bile ratio observed with phenobarbital pretreatment (from 3.4 to 1.7) was attributed to the liver's phenobarbital-enhanced ability to destroy many of the induced free-radical adducts. These results are supported by findings in bile duct-cannulated rats and in perfused rat liver systems, where spin-trapped free-radical adducts were observed in bile, but not in blood or urine (Hughes et al. 1991).

As noted above, within 6 hours of intraperitoneally injecting rats or gerbils with 128–159 mg/kg of carbon tetrachloride, 80–90% of the administered dose was expired as unchanged carbon tetrachloride, while less than 1% was expired as CO_2 (Cai and Mehendale 1990; Mehendale and Klingensmith 1988; Young and Mehendale 1989). After rats were injected intraperitoneally with 3 mL carbon tetrachloride per kg body weight, volatile carbonyl compounds released into expired air over 24 hours were evaluated by gas chromatography (Dennis et al. 1993). Injected rats exhaled significantly higher levels of acetone and a compound tentatively identified as formyl chloride than control rats; the amounts of acetaldehyde and formaldehyde were not significantly different in the two groups.

3.4.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen et al. 1987; Andersen and Krishnan 1994). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parameterization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) are adequately described, however, this simplification is desirable because data are often unavailable for

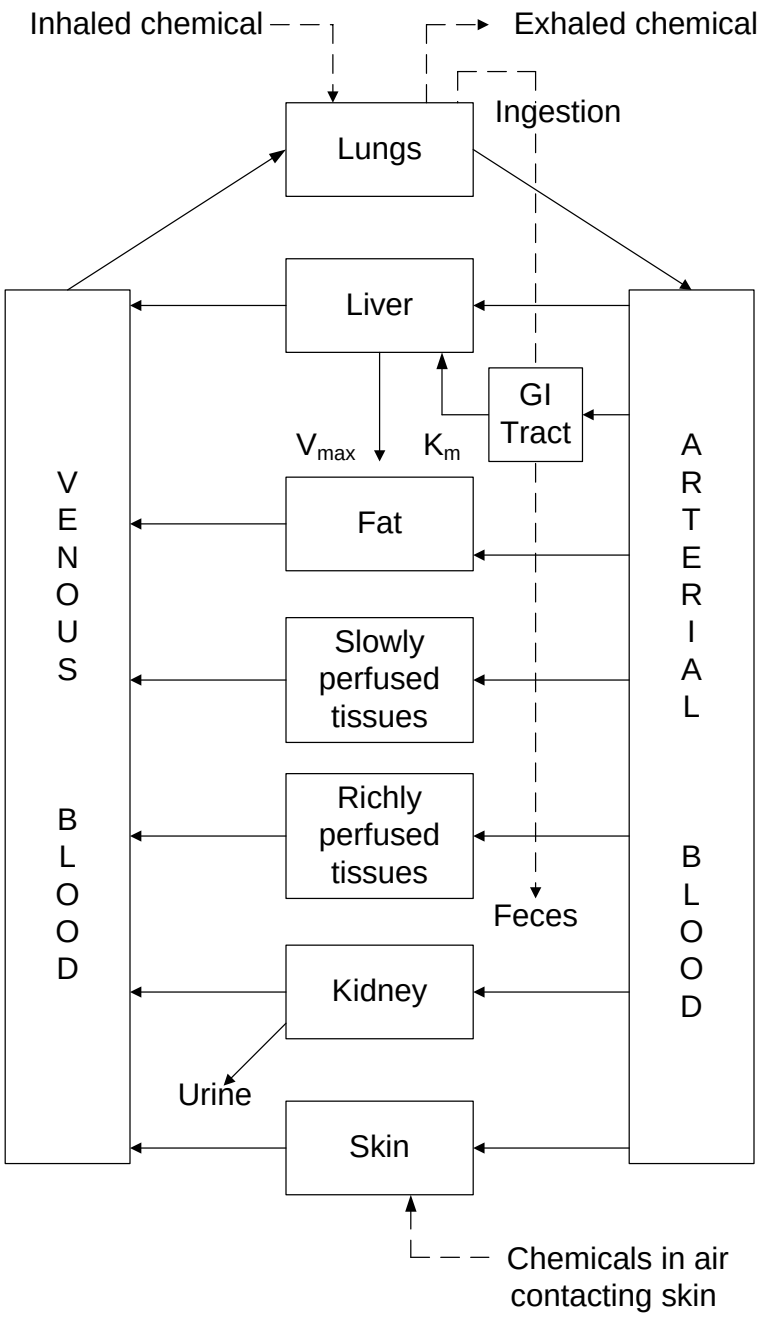
many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-4 shows a conceptualized representation of a PBPK model.

If PBPK models for carbon tetrachloride exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

A detailed physiologically based pharmacokinetic model (Figure 3-5) has been developed that describes the metabolism of carbon tetrachloride following inhalation exposure (Paustenbach et al. 1988). The model was based on and validated against a previous study in rats in which 1–2 weeks of inhalation exposure to 100 ppm ^{14}C-labeled carbon tetrachloride for 8–11.5 hours/day, 4–5 days/week apparently resulted in 40–60% of the absorbed dose being metabolized (Paustenbach et al. 1986a). The model incorporated partition characteristics of carbon tetrachloride (blood:air and tissue:blood partition coefficients), anatomical and physiological parameters of the test species (body weight, organ weights, ventilation rates, blood flows), and biochemical constants (V_{max} and K_m) for carbon tetrachloride metabolism. Rat and human parameters used in the model are listed in Table 3-7. The model accurately predicted the behavior of carbon tetrachloride and its metabolites, both the exhaled unmetabolized parent compound and $^{14}CO_2$ and the elimination of radioactivity in urine and feces. In agreement with other studies (Gargas et al. 1986; Uemitsu 1986), Paustenbach et al. (1988) found that metabolism was best described as a single saturable pathway, with a V_{max} of 0.65 mg/kg/hour and a K_m of 0.25 mg/L. Metabolites were partitioned in the model to three compartments: the amounts to be excreted in the breath (as $^{14}CO_2$), urine, and feces. Of total carbon tetrachloride metabolites, 6.5% was excreted as CO_2, 9.5% was excreted in urine, and 84.0% was excreted in feces. Based on this model, the authors estimated that about 4% of initially metabolized carbon tetrachloride is converted directly to carbon dioxide and is promptly excreted, while the remainder forms adducts with proteins and other cellular molecules. These adducts are then degraded with a half-life of about 24 hours, and the products are excreted mainly in the

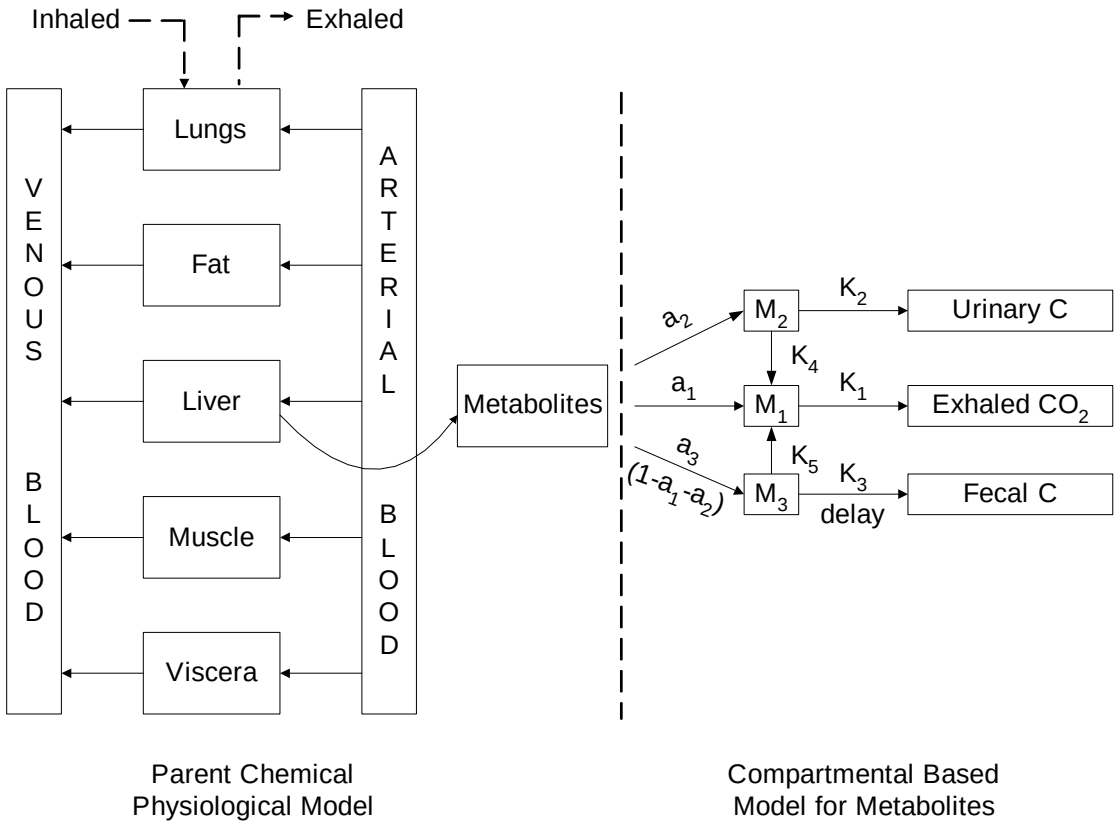
Figure 3-4. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Source: adapted from Krishnan et al. 1994

Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

Figure 3-5. Physiologically Based Pharmacokinetic Model for Inhaled Carbon Tetrachloride*



*Adapted from Paustenbach et al. 1988

Table 3-7. Parameters in PBPK Models for Carbon tetrachloride

Parameter	Rat	Human
Physiological parameters (as per Paustenbach et al. 1988)		
Body weight (kg)	0.42	70
Percentages of body weight		
Liver	4	4[a]
Fat	8	10
Rapidly perfused	5	5
Slowly perfused	74	62
Flows (L/hour)		
Cardiac output (QC)	8.15	348
Alveolar ventilation (QP)	7.91	254
Blood flow (percentages of cardiac output)		
Liver	25	25[a]
Fat	4	6
Rapidly perfused	51	51[a]
Slowly perfused	20	18
Partition coefficients		
Blood:air	4.52	2.64
Liver:blood	3.14[b]	3.14[a]
Fat:blood	79.4	79.4[a]
Rapidly perfused:blood	3.14[c]	3.14[a]
Slowly perfused:blood	1	1[a]
Metabolism		
Vmax (mg/hour)	0.35	12.72[d]
Km (mg/L)	0.25	0.25[a]

[a]Human value set equal to rat value
[b]Gargas et al. 1986
[c]Rapidly perfused blood: set equivalent to liver blood
[d]Human value scaled up using allometric equation $V_{max} = V_{maxc} \times (\text{body weight})^{0.7}$ using V_{maxc} for rat = (maximum rate of metabolism of 0.65 mg/hour-kg body weight) and human body weight of 70 kg

urine and feces, with small amounts eliminated as carbon dioxide. The amount of carbon tetrachloride metabolized is limited by the saturable enzyme system, with high exposures (e.g., 100 ppm) leading to saturation within a short time. Following cessation of exposure, considerable metabolism may occur as carbon tetrachloride emerges from fatty tissue. The model successfully described elimination using a V_{max} of 0.65 mg/kg/hour and a K_m of 0.25 mg/L. The model was scaled up to predict the expected behavior of carbon tetrachloride in monkeys and humans. The results were consistent with data collected by McCollister et al. (1951) and Stewart et al. (1961). The earlier study by Paustenbach et al. (1986) showed that rats did not have significant day-to-day accumulations in the blood or fat following repeated exposure to 100 ppm for 8 or 11.5 hours/day; this was accurately described in the model. In contrast, humans exposed to 5 ppm for 8 hours/day would be expected to show day-to-day increases in fat because of physiological differences.

Thrall et al. (2000) adapted the model of Paustenbach et al. (1988) to compare the metabolism of carbon tetrachloride in male rats, mice, and hamsters exposed to 40–1,800 ppm in a recirculating closed-chamber gas-uptake system. For each species, an optimal fit of the uptake curves was obtained by adjusting the metabolic constants V_{max} (capacity) and K_m (affinity) using the model. The mouse had a slightly higher capacity and lower affinity for metabolizing carbon tetrachloride than the rat, whereas the hamster had a higher capacity and lower affinity than either the rat or mouse. A comparison of V_{max}/K_m normalized for milligrams of liver protein (L/hour/mg) indicated that hamsters metabolize more carbon tetrachloride than rats or mice. The species comparisons were evaluated against toxicokinetic studies conducted in animals exposed by nose-only inhalation to 20 ppm ^{14}C-labeled carbon tetrachloride for four hours. Rats eliminated a lower fraction of the dose as metabolites and more as parent compound compared to mice or hamsters. The use of the model was expanded to include *in vitro* constants using liver microsomes from rat, mouse, hamster, and human in order to estimate *in vivo* metabolic rates for humans: a V_{max} of 1.49 mg/hour/kg body weight and a K_m of 0.25 mg/L. Normalizing the rate of metabolism (V_{max}/K_m), the rate of metabolism differed across species, with hamster > mouse > rat > human.

Yoshida et al. (1999) estimated rates of absorption of carbon tetrachloride and three trihalomethanes in low-level inhalation exposures by rats using a pharmacokinetic analysis. A three-compartment model, consisting of a tank with barium chloride to trap the chemical, the exposure chamber, and the rat, was employed for carbon tetrachloride, which was injected into the chamber. The model estimated that the amounts of carbon tetrachloride metabolized by rats in μmol/hour/kg were 0.000053, 0.0053, and 0.53 for exposures at 1 ppb, 10 ppb, and 10 ppm, respectively.

Semino et al. (1997) adapted the model of Paustenbach et al. (1988) to develop a PBPK model to describe the oral uptake of carbon tetrachloride administered to male Fischer 344 rats in corn oil or 0.25% Emulphor, an aqueous vehicle. The gastrointestinal model used a series of subcompartments with an absorption constant (K_a, L/hour), a bioavailability term (A, unitless), and a compartment emptying time (T, hours). The model was optimized by varying the values of the constants for the experimental data. Higher values of K_a and A were needed to fit data from aqueous gavage compared to that for corn oil. The model provided precise fits of multipeak blood and exhaled breath carbon tetrachloride concentration-time profiles. A pulsatile pattern noted following corn oil gavage was attributed to discontinuous emptying of the stomach into the small intestine. Initial absorption of the bolus occurs rapidly in the stomach, especially for aqueous vehicles; subsequently, stomach absorption slows and uptake from the small intestine determines the absorption profile.

Gallo et al. (1993) developed a PBPK model for blood concentration of carbon tetrachloride in rats following intravenous delivery in aqueous polyethylene glycol 400. Subsequently, absorption input functions were added to the model to describe blood concentration profiles resulting from administration of 25 mg carbon tetrachloride per kg body weight alone, in aqueous vehicles (water or 0.25% Emulphor emulsion), or in corn oil. Absorption was 91.9% for administration in water, 85.4% in Emulphor, 62.8% for the pure compound, and 93.1% for administration in corn oil. A pulsatile pattern was obtained for absorption in corn oil.

Andersen et al. (1996) developed a pharmacokinetic model to calculate the concentration of carbon tetrachloride in microsomal suspensions from male Fischer 344 rats under anerobic conditions. Dose-response curves revealed a nonlinear, biphasis appearance of trichloromethane. One experiment compared microsomes from fasted or unfasted rats; fasting did not alter the shape of the dose-response curve, but increased the production of trichloromethane in microsomes.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Absorption. As a small volatile haloalkane, carbon tetrachloride diffuses passively across cell membranes, leading to rapid absorption from the lungs and gastrointestinal tract into the circulatory system (Sanzgiri et al. 1995, 1997). Pulmonary absorption is ventilation limited.

Distribution. Being somewhat lipophilic, absorbed carbon tetrachloride diffuses from the blood to the liver, kidney, brain, and other organs and accumulates in adipose tissue. Following absorption by the gastrointestinal tract, a first-pass effect is apparent through the liver, where carbon tetrachloride is biotransformed and adducts are formed from reactive metabolites binding to cell macromolecules. Clearance of unmetabolized carbon tetrachloride is limited by passive diffusion; the rate of clearance is slowest for adipose tissue compared to internal organs (Benson et al. 2001; Sanzgiri et al. 1997). Quantitative differences between maximal tissue concentrations of carbon tetrachloride in the kidney or liver occur following administration of equivalent absorbed doses (179 mg/kg) by inhalation, oral bolus dosing, or gradual gastric infusion (Sanzgiri et al. 1997). Inhalation exposure resulted in similar values for kidney (25 μg/g) and liver (20 μg/g). Delivery of carbon tetrachloride as a single bolus can exceed first-pass hepatic and pulmonary elimination, resulting in higher blood levels and more severe hepatic injury compared to gradual delivery of the same dose over a longer period of time (Sanzgiri et al. 1997); bolus delivery resulted in a maximal tissue concentration for the kidney (14 μg/g) that is only 24% of the hepatic value (58 μg/g). Gastric infusion of the equivalent dose over 2 hours resulted in much lower tissue concentrations, with the kidney value (4 μg/g) 8 times higher than the value for the liver (0.5 μg/g). These results suggest that gradual oral intakes, such as might occur from contaminated drinking water, might result in less hepatotoxicity than equivalent exposure by bolus dosing or inhalation.

Metabolism. Carbon tetrachloride is primarily metabolized in tissues that express CYP2E1. The metabolic pathways are described in detail in Section 3.4.3 and depicted in Figure 3-3.

Excretion. In humans and animals, carbon tetrachloride is eliminated by passive diffusion primarily through exhaled breath, with a smaller fraction eliminated in urine and feces (Benson et al. 2001; Thrall et al. 2000).

3.5.2 Mechanisms of Toxicity

Unmetabolized carbon tetrachloride, as a volatile halogenated alkane, depresses the central nervous system. All other toxic effects of carbon tetrachloride are related to its biotransformation catalyzed by cytochrome P-450 dependent monooxygenase, specifically CYP2E1 (Azri et al. 1991; Hughes et al. 1991; Lindros et al. 1990; Raucy et al. 1993; Wong et al. 1998; Zangar et al. 2000). The liver and kidney (especially in humans) are especially vulnerable because of the abundance of CYP2E1, which is also present in the respiratory and nervous systems, and various isoforms of CYP3A (Haehner et al. 1996; Koch et al. 2002; Martin et al. 2003; Warrington et al. 2004; Wauthier et al. 2004). Considerable data

are available for hepatic toxicity, but similar cellular damage would be expected in other tissues with a high abundance of CYP2E1. There is considerable evidence that hepatic injury produced by carbon tetrachloride is mediated by two major processes resulting from bioactivation in the endoplasmic reticulum and mitochondria of centrilobular hepatocytes, which have the highest concentration of CYP2E1 (Buhler et al. 1992; Raucy et al. 1993): haloalkylation of cellular macromolecules by reactive metabolites such as trichloromethyl free radical or trichloromethyl peroxyl free radical (Link et al 1984; Mico and Pohl 1983; Poyer et al. 1980; Recknagel et al. 1977; Slater et al. 1986) and lipid peroxidation, which impairs cellular functions dependent on membrane integrity (Benedetti et al. 1982; Comporti 1985; Lee et al. 1982; Slater 1981; Slater and Sawyer 1970; Tribble et al. 1987; Weber et al. 2003). Both haloalkylation and lipid peroxidation contribute to loss of cellular functions and subsequent cell death as discussed in greater detail in the following paragraphs. In response to parenchymal cell damage, perisinusoidal cells may be stimulated to release extracellular matrix proteins (type-I collagen) that contribute to hepatic fibrogenesis, which is largely mediated by hepatic macrophages (Kupffer cells) (Belyaev et al. 1992; Ishiki et al. 1992; Johnson et al. 1992; Luckey and Petersen 2001; Muriel and Escobar 2003). Kupffer cells activated by carbon tetrachloride release tumor necrosis factor-alpha (TNF-alpha), nitric oxide, transforming growth factor-beta (TGF-beta) (Date et al. 1998), and interleukins (IL)-1, -6, and -10 (Weber et al. 2003). TNF-alpha elicits an inflammatory response and may generate aptoptosis or contribute to the development of steatosis in heptocytes (Morio et al. 2000); however, impaired secretion of lipoproteins, possibly because of lipid peroxidation, has also been proposed as a mechanism for steatosis (Boll et al. 2001c). TNF-alpha may also stimulate genes involved in hepatic mitogenesis (Bruccoleri et al. 1997). TNF-alpha is also responsible for the activity of hepatocyte nuclear factor 1 (HNF-1) in down-regulating genes for organic anion transporters (*Ntcp*, *Oatp1*, and *Oatp2*) that operate in the basolateral domain of parencymal cells following carbon tetrachloride treatment (Geier et al. 2003). Nitric oxide modulates intra-hepatic vascular tone in normal rats (Loureiro-Silva et al. 2003) and generally protects against apoptopic tissue damage (Muriel 1998), but it can also react with the O_2^- radical (formed during carbon tetrachloride-induced oxidative stress) to form an aggressive peroxynitrite radical, resulting in more severe hepatic injury (Morio et al. 2001; Weber et al. 2003). Inhibition of Kupffer cell activation with gadolinium prevented hepatic lipid peroxidation and histopathology produced by carbon tetrachloride (Muriel et al. 2001). Interleukin-6 pathways that use the signal transducer gp130 have been proposed as protective against the progression of carbon tetrachloride-induced fibrosis (Streetz et al. 2003). As shown by the results of experiments with IL-10 knockout mice, IL-10 reduces neutrophilic infiltration (inflammation) following injury with carbon tetrachloride and limits the proliferative response of hepatocytes and the development of fibrosis during the recovery phase (Louis et al. 1998). TGF-beta1 promotes hepatic fibrogenesis following carbon tetrachloride treatment, as shown

by the inhibition of fibrosis by peptides that block the cytokine's Type III receptor (Ezquerro et al. 2003). Lipid peroxidation may be at least partially independent of cytochrome P-450, as iron-dependent peroxidation occurred in cultured mammalian cells even in the presence of P-450 inhibitors (Dickens 1991). While carbon tetrachloride-induced liver damage was mitigated by treatment with allopurinol, an inhibitor of xanthine oxidase (a free radical-generating enzyme), prolonged administration of the free radical scavenger superoxide dismutase actually aggravated hepatocellular damage (Dashti et al. 1992). Some inflammatory processes in the liver following carbon tetrachloride treatment appear to be mediated by the activation of prothrombin by the prothrombinase complex (Mizuguchi et al. 2002). In acute injury from carbon tetrachloride, hydrolytic enzymes such as calpain are released from dying hepatocytes and are activated by the higher calcium levels in the extracellular space (Limaye et al. 2003). As a result, the activated proteases begin to hydrolyze proteins in neighboring cells, leading to a progression of the lesion. Necrotic responses in the liver to carbon tetrachloride are apparently mediated by the Cdk inhibitor p21, as shown by an absence of necrosis in p21-knockout mice treated with the chemical (Kwon et al. 2003).

Overall responses of different organs to carbon tetrachloride will be mediated, in part, by variations in gene expression patterns. DNA array studies in rodents demonstrated slightly different and dose-related patterns of gene expression/repression in the liver and kidney; activated genes involved heat shock proteins, oxidative stress, and DNA damage responses (Bartosiewicz et al. 2001; Fountoulakis et al. 2002; Jessen et al. 2003; Kier et al. 2004). In human hepatoma G2 cells, treatment with carbon tetrachloride increased expression and activity of interleukin-8 (IL-8), which *in vivo* directs the migration of neutrophils, resulting in release of reactive oxygen species (Holden et al. 2000). Injection of carbon tetrachloride into rats activated the expression of c-fos and c-jun within 30 minutes and also increased in hepatic nuclei the levels of the transcription factor NF-kB, which regulates transcription related to inflammation, apoptosis, and regenerative processes (Gruebele et al. 1996). Suppression of CYP3A1 and activation of multiple drug resistance gene1 (MDR1) were observed in livers of Sprague-Dawley rats following acute exposure (Kier et al. 2004). Gene expression changes associated with fibrosis (see below) may persist for weeks after cessation of treatment (Jiang et al. 2004).

Hepatic microsomal lipid peroxidation damages cellular functions by disturbing the integrity and hence the function of membranes and by covalent binding of reactive intermediates. The trichloromethyl radical is sufficiently reactive to bind covalently to CYP2E1, a process sometimes referred to as the "suicidal inactivation" of CYP2E1 (De Groot and Haas, 1981; Fernandez et al. 1982; Fujii 1997; Manno et al. 1988, 1992). It is also possible that reactive intermediates formed during the process of lipid peroxidation contribute to the loss of CYP2E1, but some *in vitro* studies have indicated that carbon tetrachloride-

induced lipid peroxidation is not required for the inactivation of CYP2E1 (Dai and Cederbaum 1995; De Groot and Haas 1980). Nevertheless, it is still not clear how these initial events are related to subsequent triglyceride accumulation, polyribosomal disaggregation, depression of protein synthesis, cell membrane breakdown and eventual death of the hepatocytes. Carbon tetrachloride can inhibit triglyceride secretion from hepatocytes in the absence of lipid peroxidation, and polyribosomal dissociation and decreased protein synthesis can occur when no ^{14}C-labelled carbon tetrachloride has been incorporated into ribosomal fractions (Waller et al. 1983). When rats were pretreated with a chemical that reduced lipid peroxidation by 85%, only small recoveries from carbon tetrachloride-induced decreases in hepatocellular viability, cytochrome P-450 content, aniline hydroxylase activity, and carbon tetrachloride metabolism capacities were observed (Kostyuk and Potapovich 1991). This suggests that free radical binding to critical cellular macromolecules (e.g., microsomal oxidation system enzymes) may be more critical for these effects than lipid peroxidation. On the other hand, inhalation exposure to carbon tetrachloride produced a direct correlation between lipid peroxidation and proline hydroxylase (a collagen biosynthetic enzyme) in rats, and dietary zinc supplementation was associated with decreases in lipid peroxidation, collagen deposition, and proline hydroxylase activity, together with an increase in collagenase activity (Camps et al. 1992). Carbon tetrachloride-induced lipid peroxidation apparently requires the presence of Fe^{2+} ions, as demonstrated by the inhibitory effect of the iron-chelating agent deferrioxamine on lipid peroxidation and hepatotoxicity in rats (Younes and Siegers 1985). One product of lipid peroxidation, 4-hydroxynonenal, has been shown to act as a pro-fibrogenic stimulus following acute hepatic injury from carbon tetrachloride (Zamara et al. 2004).

Intrinsic tissue levels of antioxidants such as glutathione influence the degree to which oxidative damage progresses following exposure to carbon tetrachloride. In 11 selected human cells types, steady-state levels of oxidative DNA base modifications (e.g., 8-hydroxyguanine) were inversely proportional to intrinsic glutathione levels (Will et al. 1999). An age-related decline in the activity of nuclear factor erythroid2-related factor (Nrf2), the factor regulating the transcription of gamma-glutamylcysteine ligase (GGCL), is the ultimate cause of the age-related decline in hepatic glutathione levels in rats (Suh et al. 2004). The hepatic activity of GGCL, which synthesizes gamma-glutamylcysteine, a precursor to glutathione, is 54.8% lower in old (24-month) rats compared to young (3-month) rats, resulting in a 35% decline in glutathione content in older rats. Agents such as buthionine sulphoximine, which inhibit GGCL, also deplete glutathione levels (Edgren and Revesz 1987). Conversely, S-adenosylmethionine (SAM), which is required for the synthesis of precursors to glutathione (homocysteine and cysteine), is also depleted by liver injury and its loss is exacerbated by the concomitant inactivation of SAM synthetase (Gasso et al. 1996). Exogenous administration of SAM or cysteine reduced carbon

tetrachloride-induced liver injury by the increase in glutathione levels (De Ferreyra et al. 1974; Gasso et al. 1996). Reduced glutathione levels concomitant with renal histopathology have also been demonstrated in the kidney of rats injected with carbon tetrachloride (Dogukan et al. 2003; Ozturk et al. 2003).

Another factor that may be of importance in carbon tetrachloride-induced hepatotoxicity is the perturbation of normal cellular calcium homeostasis following exposure. A number of studies have reported data that suggest carbon tetrachloride exposure inhibits the capacity of the hepatocyte endoplasmic reticulum or microsomal fraction to sequester (or keep sequestered) calcium, under either *in vivo* (Kodavanti et al. 1993; Long and Moore 1986a; Long et al. 1989; Lowrey et al. 1981b; Moore 1980; Moore et al. 1976) or *in vitro* (Long and Moore 1987; Long et al. 1989; Lowrey et al. 1981a; Srivastava et al. 1990; Waller et al. 1983) exposure conditions. This inhibition of sequestration capacity is considered to be a key contributor to the rise in cytosolic calcium concentration that is generally observed following carbon tetrachloride exposure (e.g., Kodavanti et al. 1990b, 1993; Long and Moore 1987), and that is postulated to play a central role in the induced cytotoxicity. The suppression of calcium uptake by microsomes occurred in the liver, (but not the kidney) of rats receiving a single oral dose of 2,500 mg/kg carbon tetrachloride (Moore et al. 1976). While some *in vivo* (Long and Moore 1986a) and *in vitro* (Srivastava et al. 1990) data suggest that carbon tetrachloride intoxication actually promotes the release of calcium to the cytosol from the endoplasmic reticulum or microsomes, other *in vivo* studies with carbon tetrachloride alone (Yamamoto 1990b) or in conjunction with chlordecone (Agarwal and Mehendale 1984a, 1984b, 1986) indicate that microsomal calcium content in fact rises, though generally to a lesser extent than cytosolic or total calcium content. Such microsomal increases presumably occur despite diminished calcium sequestration capacity. In isolated hepatocytes, immediate (<1 minute) alterations in calcium sequestation following treatment have been attributed to the solvent effect of unmetabolized carbon tetrachloride (Hemmings et al. 2002). It should be noted that another *in vitro* study found that membrane effects (membrane fusion) only occurred at concentrations that are unlikely to be achieved during inhalation exposure, but might occur following bolus gavage dosing at high levels (Johnston and Kroening 1998).

Studies have indicated that increased intracellular calcium may mediate cytotoxicity by activating phospholipase A2 (Chiarpotto et al. 1990; Glende and Recknagel 1991, 1992; Simon et al. 1986; van den Bosch et al. 1990), which might contribute to irreversible plasma membrane damage. Lipid damage from phospholipase A2 may result from increased lipid hydrolysis and from the initiation of the arachidonic acid cascade that generates toxic prostanoids (Basu 2003; Glende and Pushpendran 1986). Elevated

phospholipase A2 activity has been detected in the renal cortex and medulla of rats with carbon tetrachloride/phenobarbital-induced hepatic cirrhosis (Niederberger et al. 1998). Elevated intracellular calcium may also be associated with elevated levels of phosphorylase and altered intracellular levels and distribution of calmodulin (Kodavanti et al. 1990), but was reported not to result in any DNA degradation—a potential result of calcium-activation of endonuclease activity (Long et al. 1989).

The finding that carbon tetrachloride is converted to reactive metabolites that bind to nuclear protein, lipids, and DNA may be relevant to the understanding of carbon tetrachloride carcinogenicity. Binding of radiolabel to liver cytoplasmic and nuclear proteins was found in Wistar rats and Swiss mice dosed with ^{14}C-carbon tetrachloride (Rocchi et al. 1973). Pretreatment of the animals with 3-methylcholanthrene (an inducer of cytochrome P-450 IA [P-448]) resulted in ^{14}C binding to hepatic DNA of mice, but not rats. Similarly, Diaz Gomez and Castro (1980a) found significantly greater ^{14}C binding to the liver DNA of A/J mice than to that of Sprague-Dawley rats given a tracer dose of ^{14}C-carbon tetrachloride. A/J mice are among the most susceptible of strains tested with respect to liver tumor induction by carbon tetrachloride. Administration of a high dose (3,200 mg/kg) of ^{14}C-carbon tetrachloride, having the same total radioactivity as the tracer dose, resulted in much more intensive binding to hepatic DNA. Presumably, the fewer reactive metabolites formed from the tracer dose react primarily with microsomal lipids and proteins in close proximity to their formation. With the higher dose, more ^{14}C-carbon tetrachloride can apparently reach the nucleus and be metabolically activated there, subsequently reacting with nuclear lipids, proteins, and DNA. This scenario receives support from the finding that highly purified rat liver nuclear preparations were able to anaerobically activate ^{14}C-carbon tetrachloride in the presence of an NADPH generating system (Diaz Gomez and Castro 1980b). Under microsome-mediated aerobic conditions, it was observed that ^{14}C-carbon tetrachloride bound more to histone than to nonhistone chromosomal proteins from livers of B6C3F$_1$ mice (Oruambo and Van Duuren 1987). These findings may be relevant to the understanding of carbon tetrachloride hepatocarcinogenicity, since reactive metabolites of carbon tetrachloride appear capable of binding to targets of putative relevance to cancer induction (chromosomal DNA and nucleosome proteins), and may even be generated within the nucleus itself. Since lipid peroxidation products such as malonaldehyde also have the ability to form adducts with DNA (Chaudhary et al. 1994; Chung et al. 2001; Wacker et al. 2001), it is possible that the genotoxic effect of carbon tetrachloride is partly indirect. Malonaldehyde-initiated tumors have been reported in Swiss mice (Shamberger et al. 1974). It is also worth noting that data from a variety of congenic mouse strains suggest that both the toxicity of, and recovery from, carbon tetrachloride exposure are under genetic control (an Ah gene, and H-2 genes) (Bhathal et al. 1983; Biesel et al. 1984).

Results of chronic bioassays in rats and mice exposed orally or by inhalation indicate that hepatocellular carcinomas are induced at hepatotoxic doses, suggesting that there may be a threshold for carcinogenicity of carbon tetrachloride (Japan Bioassay Research Centre 1998; NCI 1976a, 1976b, 1977). The results of these chronic rodent studies are consistent with the idea that hepatic carcinogenicity directly related to the increase in cellular replication that occurs in response to hepatocyte lethality. Enhanced cellular replication increases the possibility that unrepaired DNA errors will become fixed mutations, possibly resulting in an initiated preneoplastic cell. Exposures at levels lower than those eliciting hepatic regeneration would not be expected to result in hepatic carcinogenicity.

Interesting data from other studies illustrate that the hepatotoxic effects of carbon tetrachloride (or carbon tetrachloride plus chlordecone) depend not merely on its metabolic activation, but also to a substantial degree on the liver's hepatocellular regenerative capacity (e.g., Mehendale 1990, 1991, 1992). For example, the auto protection conferred by a low nontoxic dose of carbon tetrachloride against the toxic effects of a subsequent high dose seem not to be completely accounted for by mere destruction of cytochrome P-450 activation capacity, but appear also to involve the early (2–6 hours after pretreatment) stimulation of hepatocellular regeneration (Rao and Mehendale 1991; Thakore and Mehendale 1991). This early, low-dose stimulation, which leads to much greater hepatocellular regenerative activity (DNA-synthesis and mitosis) following the high-dose exposure, and the autoprotection phenomenon are both inhibited by a colchicine-induced mitotic block (Rao and Mehendale 1991, 1993). It has been hypothesized that the low dose of carbon tetrachloride and/or the resulting minimal injury induces hepatocytes into the cell cycle from an arrested G_2 state (Calabrese et al. 1993). Further, partial hepatectomy in rats has been shown to confer resistance to carbon tetrachloride-induced hepatotoxicity, presumably via enhanced regenerative capacity, as hepatic uptake and metabolism of carbon tetrachloride was not significantly altered (Young and Mehendale 1989). The particular sensitivity of gerbils to carbon tetrachloride-induced hepatotoxicity appeared related not only to extensive bioactivation, but also to a sluggish hepatocellular regenerative and tissue repair response, and was mitigated by partial hepatectomy that stimulated this response in the absence of any significant effect on carbon tetrachloride bioactivation or induced lipid peroxidation (Cai and Mehendale 1990, 1991a, 1991b). Finally, in rats, pretreatment with nontoxic levels of chlordecone has been shown to substantially potentiate the hepatotoxicity of low doses of carbon tetrachloride without affecting its hepatic metabolism to a similarly significant degree, whereas phenobarbital pretreatment induced greater bioactivation, but less hepatotoxicity (Mehendale and Klingensmith 1988; Young and Mehendale 1989). This chlordecone potentiation phenomenon has been attributed to its inhibitory effect on the level of hepatocellular regeneration and tissue repair normally induced by low-dose carbon tetrachloride, with death resulting from hepatic failure and hepatic

encephalopathy (renal toxicity was not affected) (Kodavanti et al. 1992; Soni and Mehendale 1993). Where chlordecone cannot inhibit this regenerative response, as in cultured rat hepatocytes (Mehendale et al. 1991) or gerbils (Cai and Mehendale 1990), it does not potentiate cellular or hepatic toxicity.

The signaling factor leptin apparently plays a role in the initiation of hepatic regenerative processes following acute injury by a sublethal dose of carbon tetrachloride (Leclercq et al. 2003). Hepatic changes occurring in mice treated with carbon tetrachloride (in temporal order) include induction of nuclear factor kappaB (NF-kB) and interleukin-6 (IL-6) at the time of G1/S transition, increased DNA binding by STAT3 (signal transducer and activator of transcription), induction of cyclin D1 expression consistent with an increase in mitosis, and a time-dependent increase in tumor necrosis factor (TNF) consistent with the appearance of necrosis. NF-kB also regulates genes involved in inflammation, apoptosis, proliferation, and regeneration in the liver (Gruebele et al. 1996). Mice (*ob/ob)* transgenic for the loss of expression of leptin and treated with carbon tetrachloride had an exaggerated expression of STAT3 and NF-kB, impaired activation of TNF and IL-6 release, failure of induction of cyclin D1, and reduced hepatocyte proliferation. Exogenous leptin restored the regenerative capacity of *ob/ob* mice. Hepatic regeneration following acute injury by carbon tetrachloride is mediated by the type 1 TNF receptor, but not the type 2 receptor, in mice (Yamada and Fausto 1998).

Fibrotic processes in the liver following carbon tetrachloride treatment are modulated by the adipocytokine adiponectin (Kamada et al. 2003). Repeated (twice weekly) intraperitoneal dosing with 1,594 mg/kg but not 478 mg/kg carbon tetrachloride significantly reduced plasma concentrations of adiponectin. Although showing the same degree of initial hepatic injury (serum ALT, inflammation) as wild type mice following injection with carbon tetrachloride, adiponectin-knockout mice showed more extensive liver fibrosis (hydroxyoproline content) and enhanced expression of TGF-beta1 and connective tissue growth factor (CTGF) compared to wild type mice. Replacement dosing with adiponectin reduced hepatic fibrosis. The effect of adiponectin on cultured hepatic stellate cells stimulated with platelet-derived growth factor-BB (PDGF-BB) was to inhibit proliferation and migration and counteract the TGF-beta1-induced activation of TGF-beta1 and CTGF genes by interfering with the nuclear translocation of Smad2. Overall, a reduction of adiponectin levels following carbon tetrachloride treatment would be expected to foster fibrotic processes in the liver.

Telomere shortening resulting in chromosomal instability has been associated with hepatocellular carcinoma in humans, with loss of regenerative capacity in chronic liver injury. Intraperitoneal injection of carbon tetrachloride into wild type mice increased the initiation of hepatic foci and the development of

hepatocellular carcinoma (Farazi et al. 2003). The number and size of hepatic nodules were significantly lower in treated mice that were transgenic for aberrant telomerase. The authors suggest that telomere dysfunction impedes the progression to malignancy. Estradiol protected against telomere shortening, fibrosis, and senescence in hepatocyes of rats injected intramuscularly with carbon tetrachloride (Sato et al. 2004). The authors attributed the protective effect of estradiol on its transactivation of the telomerase gene.

3.5.3 Animal-to-Human Extrapolations

Patterns of toxicity and metabolism of carbon tetrachloride in laboratory animals are very similar in humans and animals. In both, similar effects are observed in the major target organs, the liver and kidney, as well as in the nervous system during acute inhalation exposures. There are some minor species differences in metabolic parameters following exposure to carbon tetrachloride. Benson et al. (2001) reported that the fraction of carbon tetrachloride (equivalents following inhalation of radiolabeled carbon tetrachloride) partitioning to the liver after a 4-hour inhalation exposure was higher in hamsters and mice than in rats, which show an immediate accumulation in fat. . Rats eliminated less radioactivity associated with metabolism and more associated with the parent compound in exhaled air than mice or hamsters. Thrall et al. (2000) estimated that humans at low inhalation concentrations metabolized less of the dose than rats, and would be less sensitive than rats at equivalent exposures; the rate of metabolism was highest in mice, followed by rat, and then humans. In humans, rats, and mice, CYP2E1 is the major enzyme responsible for bioactivation of carbon tetrachloride; thus, similar effects of reactive metabolites could be expected in rodents and humans.

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Colborn and Clement (1992), was also used in 1996 when Congress mandated the EPA to develop a screening program for “...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...”. To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), and in

1998, the EDSTAC completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

There is no reported direct effect of carbon tetrachloride on hormones in humans or animals. Fertility was reduced in an inhalation bioassay in rats, but it is not known whether the cause was hormonal disruption or a necrotic effect on the gonads (Smyth et al. 1936). Testicular degeneration, possibly resulting from necrosis, was observed in rats exposed by inhalation (Adams et al. 1952). Adrenal pheochromocytomas were induced in mice exposed to carbon tetrachloride vapor for 2 years (Japan Bioassay Research Center 1998). It is possible that catecholamine balances were affected in these animals (Landsberg and Young 1998).

It is possible that the loss of hepatic function caused by carbon tetrachloride could indirectly impair hormone metabolic processes that are regulated by the liver. Functions that could be affected by reduced liver function include inactivation of some hormones (e.g., insulin and glucagon) by proteolysis or deamination, deiodination of thyroxine and triiodothyronine, inactivation of steroid hormones (e.g., glucocorticoids and aldosterone) followed by glucuronidation, metabolism of testosterone to 17-ketosteroids and sulfonation, conversion of estrogens to estriol and estrone followed by conjugation to glucuronic acid or sulfate, and removal of circulating vasoactive substances such as epinephrine and

bradykinin (Podolsky and Isselbacher 1998). In humans, chronic liver disease not caused by carbon tetrachloride is known to result in signs of hormonal imbalance such as testicular atrophy (Podolsky and Isselbacher 1998). The development of ascites in chronic liver disease may be facilitated by the elevated levels of epinephrine (Podolsky and Isselbacher 1998).

No *in vitro* studies were located regarding endocrine disruption of carbon tetrachloride.

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6, Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many

xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

One epidemiological study reported associations between maternal exposure to carbon tetrachloride at levels higher than 1 ppb in drinking water and adverse developmental outcomes (low full-term birth weight and small for gestational age) in humans (Bove et al. 1992a, 1992b, 1995). However, the same effects were associated with exposure to trihalomethanes, which occurred at higher concentrations in drinking water. Associations between exposure and incidences of central nervous system defects, cleft-lip or cleft-palate, or heart conotruncal defects were not statistically significant (Bove et al. 1992a, 1992b, 1995; Croen et al. 1997). In general, exposure to other chemicals by the study population raises uncertainty as to the causative role of carbon tetrachloride in the observed adverse developmental effects. Animal studies did not report adverse developmental effects in the absence of maternal toxicity. No teratogenic effects (morphological anomalies) were observed in rats exposed to carbon tetrachloride by inhalation (Gilman 1971; Schwetz et al. 1974) or in rats or mice exposed by ingestion (Hamlin et al. 1993; Wilson 1954). Complete litter loss occurred in some rats given oral doses that produced clear maternal toxicity (Narotsky et al. 1997a, 1997b; Wilson 1954). It is not known whether litter loss is the result of toxicity to the fetus or to the placenta, but the critical site of injury is likely related to the abundance of cytochrome proteins that metabolize carbon tetrachloride.

An *in vitro* fertilization assay in mice reported significant adverse effects on fertilization at concentrations ≥1 mM (154 μg/mL), but not ≤0.5 mM (77 μg/mL) (Hamlin et al. 1993). These levels are significantly higher than those encountered by the general population (see Section 6.5). An assay in mice intraperitoneally injected with carbon tetrachloride found no increase in sperm head abnormalities at doses as high as 3,180 mg/kg (Topham 1990).

Fetal tissues and the placenta appear to have the capacity for bioactivating carbon tetrachloride, although the levels of cytochrome enzymes are lower than in neonates or adults (EPA 2001). Total fetal liver CYP content is a relatively constant 30% of the adult level from the end of the first trimester of gestation up to 1 year of age (EPA 2001). mRNA for CYP2E1 has been detected in human first-trimester placentas (Hakkola et al. 1996). Low levels of CYP2E1 protein have been detected in human fetal brain as early as gestational day 46, substantially increasing around day 50 (Boutelet-Bochan et al. 1997; Brzezinski et al. 1999). In the fetal liver, CYP2E1 protein was not detectable at 10 weeks of gestation, but was present at 16 weeks (Carpenter et al. 1996). Therefore, it would appear that there is a period early in gestation during which the fetal brain might be more vulnerable than the liver to the effects of carbon tetrachloride. However, no developmental studies are available that specifically examined neurological or neurobehavioral effects of exposure to carbon tetrachloride during gestation. Additionally, there is some evidence that maternal alcohol consumption induces placental CYP2E1 in humans (Rasheed et al. 1997b). If maternal alcohol exposure also increases levels of CYP2E1 in fetal tissues, the likelihood of fetal injury from exposure to carbon tetrachloride would be increased. Induction of fetal hepatic CYP2E1 by maternal ethanol consumption has been confirmed in rats (Carpenter et al. 1997). Transcription of the CYP2E1 gene in human placenta and fetal lung and kidney is regulated in part by hypermethylation of dinucleotide CG residues within the promoter (Viera et al. 1998).

Hepatic levels of CYP2E1 mRNA increase significantly during the first 24 hours after birth, largely resulting from demethylation that allows transcription to proceed (Viera et al. 1996). Major accumulations of CYP2E1 occur between 1 and 3 months of age and values comparable to those of adults are achieved sometime between 1 and 10 years of age (EPA 2001; Viera et al. 1996). Thus, children exposed to carbon tetrachloride would be expected to experience similar effects as in adults.

Fisher et al. (1997) have calculated that maternal exposure to carbon tetrachloride is likely to result in its transfer to breast milk, which would be a possible means of exposure for nursing infants.

Effects of metabolism of carbon tetrachloride on late (day 20) rat fetal hepatic microsomes have been measured *in vitro* (Cambron-Gros 1986). Fetal microsomes had the ability to metabolize the compound as evidenced by inhibition of cytochrome P-450 (to a greater degree than maternal microsomes), inhibition of calcium uptake (similar to maternal microsomes), and the increased amount of carbon tetrachloride bound to protein (less than maternal microsomes). The production of trichloromethyl radicals by fetal microsomes did not induce the membrane phospholipid peroxidation observed with maternal microsomes. The absence of lipid peroxidation in fetal liver would be expected to result in a qualitatively different pattern of hepatic toxicity following exposure to carbon tetrachloride compared to adults. The authors suggest that necrotic effects would be less in fetuses than in adults. The basis for the lack of lipid peroxidation by fetal microsomes was not determined in that study.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to carbon tetrachloride are discussed in Section 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by carbon tetrachloride are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10 “Populations that are Unusually Susceptible.”

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Carbon Tetrachloride

Measurement of parent carbon tetrachloride and its metabolites in expired air has been the most convenient way to determine exposure. Levels of 9.5 ppm carbon tetrachloride were detected in expired air of one worker who had been exposed to carbon tetrachloride vapors for several minutes (Stewart et al. 1965). In another case, expired air levels were over 2,000 ppm in a person exposed by ingestion to a pint of carbon tetrachloride mixed with methanol (Stewart et al. 1963). Levels fell below 2 ppm after 16 days. Depending on dose and length and route of exposure, the half-life of carbon tetrachloride in expired air initially appears to range from 1 to several hours, later lengthening to 40–>85 hours. Measurement of carbon tetrachloride in blood has also been used as an indicator of exposure.

Covalent adducts between reactive carbon tetrachloride metabolites (trichloromethyl radical) and cellular protein, lipids, and nucleic acids are known to occur. Although measurements of such adducts may provide data on past exposure, the method's overall usefulness in assessing exposure in the general population is severely limited since it requires the use of radiolabeled carbon tetrachloride. Further, metabolite compounds and their adducts may originate in ways other than from carbon tetrachloride, or they may undergo reduction and thus require some reoxidation procedure prior to being detectable by *in vivo* spin trapping techniques (Sentjure and Mason 1992).

3.8.2 Biomarkers Used to Characterize Effects Caused by Carbon Tetrachloride

As discussed in Section 3.2, the effects that are most often observed in humans exposed to carbon tetrachloride are liver and kidney injury and central nervous system depression. Exposure levels leading to these effects in humans are not well-defined. The threshold for central nervous system effects following exposures of 8 hours or more is probably in the range of 20–50 ppm (Elkins 1942; Heimann and Ford 1941; Kazantzis and Bomford 1960). On the other hand, kidney and liver effects can occur following exposure (15 minutes to 3 hours) to vapor concentrations of 200 and 250 ppm, respectively (Barnes and Jones 1967; Norwood et al. 1950). These exposures correspond to an absorbed dose of approximately 100–200 mg/kg.

Detection of liver injury has commonly been associated with alterations in serum levels of certain hepatic enzymes and proteins. Elevation in bilirubin levels following exposure (Barnes and Jones 1967) has been detected in humans, as have decreased serum levels of secreted liver proteins (e.g., albumin and fibrinogen) (Ashe and Sailer 1942; McGuire 1932; New et al. 1962; Norwood et al. 1950; Straus 1954). Elevations in serum levels of enzymes (alkaline phosphatase and gamma-glutamyltransferase) released from damaged hepatocytes have been reported in occupational exposures above 1 ppm lasting months to years (Tomenson et al. 1995). Similar enzyme elevations were observed following acute-, intermediate-, and chronic-duration exposures to carbon tetrachloride in animals (Bruckner et al. 1986; Hayes et al. 1986; Japan Bioassay Research Center 1998; Sakata et al. 1987). Typically, ALT, AST, alkaline phosphatase, and LDH have been monitored, but these are also produced in nonhepatic tissues. Ikemoto et al. (2001) investigated serum levels of several urea-cycle enzymes that are more exclusively found in the liver: liver-type arginase (ARG), ornithine carbamoyltransferase (OCT), and arginosuccinate synthase (AS). After rats were injected with carbon tetrachloride, serum ARG levels were immediately elevated at the first 15-minute timepoint and within 30 minutes, were about 45-fold higher than normal; after 300 minutes, the increase in serum ARG levels had not reached a plateau. All other enzymes (AST, ALT, OCT, and AS) measured had maximally 10-fold increases. The authors propose that ARG is a sensitive biomarker for acute exposure to carbon tetrachloride and attribute its pattern of appearance in serum to the fact that it is a cytosolic enzyme (having only the plasma membrane as a barrier to the extracellular compartment) and to its smaller molecular mass compared to the other enzyme biomarkers.

Yamaguchi et al. (2002) proposed that serum concentrations of regulcalcin, a Ca^{2+}-binding protein that is especially abundant in the liver but not abundant in the kidney, heart, or brain of rats, would be a sensitive measure of hepatitis following exposure to carbon tetrachloride. In rats that received 5 doses of

15,940 mg/kg at 3-day intervals, standard serum markers for AST and ALT were significantly elevated compared to controls in samples taken during the first week of exposure, but not later, whereas significant elevations in serum regucalcin were detectable both during the first week and, at lower levels, 18 and 30 days after the first exposure.

In rats acutely treated with 1,275 mg/kg carbon tetrachloride, histological evaluations of the liver showed peak scores for necrosis at 24–36 hours and for inflammation at 48 hours, with resolution largely evident by 60 hours and 4 days, respectively (Giffen et al. 2003). Detection of serum markers for necrosis and inflammation demonstrated a pattern consistent with the histological results. Serum markers of hepatic damage (AST, ALT, glutamate dehydrogenase) showed peak elevations at 36 hours and a subsequent decline. Serum haptoglobin, a marker for inflammation, peaked at 48 hours (400% over control), and after declining, was significantly elevated at 4 days but not later. The authors suggest that haptoglobin would be a sensitive marker for hepatic inflammation, since that protein is primarily synthesized by centrilobular hepatocytes, which are vulnerable to injury by carbon tetrachloride.

In the rat, carbon tetrachloride-induced liver cytolysis has been associated with elevated serum activities of glutamate dehydrogenase, sorbitol dehydrogenase, and glucose-6-phosphatase (microsomal glucose-6-phosphatase activity was decreased) (Brondeau et al. 1991, 1993), while serum procollagen III peptide was demonstrated to be a valuable indicator of liver fibrogenesis, and serum prolidase was shown to be a limited signal of accelerated liver collagen metabolism (Jiang et al. 1992). Serum immunoassay for the 7S fragment of type IV collagen may be an even more sensitive indicator of hepatic fibrosis in man (Ala-Kokko et al. 1992). Another sensitive (but nonspecific) indicator of liver injury is the serum levels of individual bile acids (Bai et al. 1992). Lipid peroxidation, increased erythrocyte membrane cholesterol/phospholipid ratio, and decreased erythrocyte ATPase activity were all associated with the onset of carbon tetrachloride-induced liver cirrhosis (Mourelle and Franco 1991). Also, lipid peroxidation accompanying carbon tetrachloride-induced hepatotoxicity has been monitored by quantitating hepatic levels of hydroperoxy- and hydroxy-eicosatetraenoic acids (Guido et al. 1993).

Renal injury has been associated with acute exposure of humans to carbon tetrachloride. Impaired renal function as evidenced by oliguria and anuria have been reported (Barnes and Jones 1967; Norwood et al. 1950). Proteinuria, hemoglobinuria, and glycosuria have also been reported in other cases involving acute exposure of humans to the compound (Forbes 1944; Guild et al. 1958; New et al. 1962; Smetana 1939; Umiker and Pearce 1953). Although acute renal failure induced in rats by carbon tetrachloride apparently did not involve activation of the circulating active renin-angiotensin system, increased

prorenin levels were associated with decreased renal function (Cruz et al. 1993). Long-term inhalation exposure to carbon tetrachloide increased the incidence and severity of chronic nephropathy in the rat, and significantly increased proteinuria levels and serum biomarkers such as blood nitrogen (Japan Bioassay Research Center 1998). These renal effects can occur following exposure to chemicals other than carbon tetrachloride.

Neurotoxicity, as evidenced by central nervous system depression, has been associated with acute exposure to carbon tetrachloride in humans. Clinical signs and symptoms that may be monitored include headache, dizziness, fatigue, and coma (Cohen 1957; Stevens and Forster 1953; Stewart et al. 1961). Impaired visual functions have also been observed (Johnstone 1948; Smyth et al. 1936; Wirtschafter 1933). It should be noted that central nervous system effects disappear rapidly as carbon tetrachloride is eliminated from the body. Therefore, they will be detectable for only relatively short periods after exposure. The neural effects are not specific to carbon tetrachloride exposure and may occur following exposure to other chemicals.

Lipid peroxidation products appearing in urine following exposure to carbon tetrachloride offer the possibility of noninvasive monitoring for hepatic damage (de Zwart et al. 1998). As measured by gas chromatography, the urinary levels in rats of the following lipid peroxidation products showed statistically significant increases over normal values within 12 hours of an intraperitoneal injection with 0.5 or 1.0 mL/kg carbon tetrachloride: formaldehyde, acetaldehyde, propanal, butanal, pentanal, hexanal, and malondialdehyde (MDA). The 0.25 mL/kg dose elicited significant increases only in acetaldehyde. The level of MDA returned to normal after 48 hours, at which time the levels of the other chemicals remained elevated. The same study found that neither coproporphyrin III nor 8-hydroxy-2'-deoxy-guanosine were suitable urinary biomarkers for exposure to carbon tetrachloride.

Metabonomics is a new technology combining high resolution nuclear magnetic resonance (NMR) and pattern recognition technology that is starting to be applied to the evaluation of *in vivo* toxicology. Robertson et al. (2000) treated rats with single intraperitoneal or oral doses of carbon tetrachloride and evaluated the changes in NMR spectra of urine as displayed by principal component analysis (PCA), a statistical method that reduces multidimensional data to a two- or three-dimensional pattern. The PCA pattern was most altered compared to the pretreatment state on the first and second days after treatment, but had returned to normal within 10 days. PCA patterns were detectable in rats treated with 0.5 mg/kg, but not in rats treated with 0.1 mg/kg.

Additional information concerning biomarkers for effects on the immune, renal, and hepatic systems can be found in the CDC/ATSDR Subcommittee Report on Biological Indicators of Organ Damage (CDC/ATSDR 1990), and on the neurological system in the Office of Technology Assessment Report on Identifying and Controlling Poisons of the Nervous System (OTA 1990).

3.9 INTERACTIONS WITH OTHER CHEMICALS

There is substantial evidence that the toxicity of carbon tetrachloride is dramatically increased by alcohols, ketones and a variety of other chemicals. Many of these might be found at hazardous waste sites also containing carbon tetrachloride. Although the precise mechanisms for this marked potentiation are not always known, it is likely that most potentiators act, at least in part, by increasing the metabolic activation of carbon tetrachloride to its toxic intermediates and metabolites, thus increasing the induced injury. Other agents may affect the toxic outcome by altering cellular regenerative and tissue repair capacities. The extent to which either or both of these mechanisms are involved in the interaction will substantially affect the relationships among induced injury, duration of toxic damage, and animal survival. Interactions with agents enhancing lipid peroxidation would be expected to increase the severity of cell injury due to increased permeability of cell membranes.

Ethanol. Alcohol (ethanol) ingestion has often been associated with potentiation of carbon tetrachloride-induced hepatic and renal injury in humans (Manno et al. 1996). In two cases in which men cleaned furniture and draperies with carbon tetrachloride, one man, a heavy drinker, became ill and died, whereas his coworker, a nondrinker, suffered a headache and nausea, but recovered quickly after breathing fresh air (Smetana 1939). Both men were subjected to the same carbon tetrachloride exposure, as they had been working in the same room for the same amount of time. In 19 cases of acute renal failure due to carbon tetrachloride inhalation or ingestion, 17 of 19 patients had been drinking alcoholic beverages at about the time of their carbon tetrachloride exposure (New et al. 1962). Many other cases of carbon tetrachloride-induced hepatic and/or renal injury associated with ethanol ingestion have been described in the medical literature (Durden and Chipman 1967; Guild et al. 1958; Jennings 1955; Lamson et al. 1928; Markham 1967; Tracey and Sherlock 1968). These clinical reports establish that occasional or frequent ingestion of alcoholic beverages can increase the danger from exposure to carbon tetrachloride at levels that otherwise do not result in significant toxicity. As ethanol is known to induce microsomal mixed-function oxidase activity in man (Rubin and Lieber 1968), the mechanism of potentiation may involve ethanol-induced enhancement of the metabolic activation of carbon tetrachloride.

Numerous studies in animals confirm that ethanol is a strong potentiator of carbon tetrachloride-induced hepatotoxicity (Ikatsu et al. 1991; Kniepert et al. 1991; Reinke et al. 1988; Sato and Nakajima 1985; Strubelt 1984; Teschke et al. 1984; Wang et al. 1997a). Ethanol administration 16–18 hours before carbon tetrachloride exposure potentiated hepatotoxicity (Cornish and Adefuin 1966; Towner et al. 1991); however, enhancement was less when ethanol was given 2 hours before carbon tetrachloride (Cornish and Adefuin 1966). This is consistent with the idea that ethanol increases carbon tetrachloride toxicity by inducing the synthesis of one or more enzymes, such as cytochrome P-450 2E1 (Castillo et al. 1992), that are involved in the metabolic activation of carbon tetrachloride; or by acting as a competitive inhibitor of carbon tetrachloride metabolism during concurrent exposure. Thus, the precise timing of exposure to each agent is likely to critically influence the observed effects. For example, a single dose of ethanol 18 hours prior to intraperitoneal administration of 1,275 mg/kg carbon tetrachloride in rats did not increase either trichloromethyl free-radical adducts or p-nitrophenol hydroxylase activity, whereas 2 weeks of dietary exposure to ethanol significantly increased the generation of trichloromethyl radicals (Reinke et al. 1988, 1992). Threshold levels also appear involved, as 14 days of 0.05–0.5 mL/kg/day ethanol did not result in a statistically significant increase in any effects of a subtoxic 20 mg/kg/day dose of carbon tetrachloride (Berman et al. 1992). Ethanol exposure intensified carbon tetrachloride toxicity in pregnant rats and caused decreased postnatal survival of offspring (Gilman 1971). For the most part, these studies involved short-term exposures to ethanol. Inhalation studies involving longer-term pretreatment exposures to ethanol (5–10 weeks) prior to carbon tetrachloride exposure raised the possibility of increased susceptibility to chronic liver injury at low doses of carbon tetrachloride that have not been shown to cause significant liver damage (Hall et al. 1990). On the other hand, when ethanol pretreatments increased in duration (30 or 52 weeks), there was a decrease in ethanol potentiation of carbon tetrachloride toxicity (Kniepert et al. 1990). Factors contributing to this diminished potentiation were not determined. It has also been reported that despite substantial potentiation of carbon tetrachloride-induced hepatotoxicity in ethanol pretreated rats, no increase in lethality was observed (Ray and Mehendale 1990). The authors speculated that this result occurred due to the treatment's concomitant stimulation of hepatic regenerative capacity—to a degree sufficient to overcome the induced injury. In addition to enhanced hepatotoxicity pretreatments with ethanol have been reported to enhance certain immunosuppressive effects of carbon tetrachloride (Kaminski et al. 1990).

Other Alcohols and Ketones. Secondary alcohols can also potentiate carbon tetrachloride hepatorenal toxicity in humans. Eighteen workers in an isopropyl alcohol packaging plant became ill after inhalation of carbon tetrachloride (Folland et al. 1976). Four of these people were hospitalized; one with liver injury, one with kidney damage, and the other two with both kidney and liver injury. Air samples taken

at the plant during a subsequent investigation revealed relatively high concentrations of isopropanol and acetone, and these were thought to play a major role in potentiation of toxicity. Potentiation of carbon tetrachloride hepatoxicity in mice by isopropanol far exceeded that caused by an equal dose of ethanol, though both exerted their maximum effect when given 18 hours before carbon tetrachloride (Traiger and Plaa 1971). In rats, isopropanol potentiated hepatic injury caused by carbon tetrachloride, but lethality was not increased because of the augmentation of hepatic tissue repair mechanisms (Rao et al. 1996). Methanol co-treatment in rats potentiated the hepatotoxicity of carbon tetrachloride by inducing CYP2E1 in rat liver (Allis et al. 1996). Methanol was found to be markedly less effective on an equimolar basis than either isopropanol or tertiary-butanol in enhancing carbon tetrachloride-induced hepatotoxicity in rats (Harris and Anders 1980). These differences likely reflect the substantially longer half-lives of the secondary and tertiary compounds (relative to their primary congeners), which makes them more potent and persistent inducers of cytochrome P-450 activities. Methanol, ethanol, isopropanol, or decanol in combination with carbon tetrachloride caused massive liver damage, but failed to increase carbon tetrachloride induced lethality. On the other hand, tert-butanol, pentanol, hexanol, and octanol not only potentiated liver damage when administered prior to carbon tetrachloride, but also significantly increased the lethal effects of carbon tetrachloride (Ray and Mehendale 1990). Thus, potentiated hepatotoxicity, as measured by various endpoints, may not be a very reliable predictor of the eventual survival outcome. Other experiments in rats demonstrated that both isopropanol and acetone (the major metabolite of isopropanol) are apparently responsible for the marked enhancement of carbon tetrachloride hepatotoxicity (Plaa and Traiger 1972). Similarly, the metabolism of 2-butanol to 2-butanone contributed to the marked ability of this alcohol to potentiate carbon tetrachloride hepatotoxicity in rats (Traiger and Bruckner 1976).

Investigations in rats indicate that ketosis, caused either by diabetes or administration of ketones, can potentiate carbon tetrachloride hepatotoxicity. Pre-treatment with methyl isobutyl ketone, acetone, or metyl ethyl ketone increased hepatotoxicity in rats treated with a single dose of carbon tetrachloride, essentially reducing the ED_{50} for carbon tetrachloride by 80, 73, or 89%, respectively (Raymond and Plaa 1995). Hepatotoxicity (fibrosis and cirrhosis) and nephrotoxicity were increased in rats exposed to both acetone and carbon tetrachloride (Charbonneau et al. 1986). Carbon tetrachloride hepatotoxicity increased in diabetic rats (Hanasono et al. 1975), while 1,3-butanediol induced ketosis and potentiated carbon tetrachloride hepatoxicity (Pilon et al. 1986). In both studies, ketosis was a better index for prediction of liver injury than glycemic status. Interestingly, the same specific form of cytochrome P-450 was reported to be induced in rats by chronic ethanol administration (Joly et al. 1977) and by diabetes (Past and Cook 1982). The bulk of available evidence suggests that elevated levels of ketone bodies

induce the enzyme system responsible for biotransformation of carbon tetrachloride to its reactive metabolites (Pilon et al. 1986). Methyl isobutyl ketone significantly increased total levels of cytochrome P-450 in rat liver microsomes (Raymond and Plaa 1995).

Phenobarbital, Metamphetamine, DDT, PBB, Chlordecone. Phenobarbital (PB) has been shown to produce a marked increase in carbon tetrachloride hepatotoxicity in rats and it is widely used to provide experimental animal models of carbon tetrachloride-induced cirrhosis (Abraham et al. 1999; Cornish et al. 1973; Garner and McLean 1969; Hocher et al. 1996; Sundari et al. 1997). This is not surprising, in that cytochrome P-450 PB-B (CYP2B1), the isozyme that can be induced at least 50-fold in rats by PB, participates in the metabolic activation of carbon tetrachloride (Vittozzi and Nastainczyk 1987). Lethal effects of carbon tetrachloride are not potentiated by even large doses of phenobarbital in spite of increased liver injury. Thus, as with the alcohols, manifestations of bioactivation capacity or hepatic injury do not appear to reliably predict the eventual survival outcome. The mechanism underlying this phenomenon appears to be the stimulation of hepatic regeneration and tissue repair. Although the early phase of hepatic regeneration was postponed from 6 to 24 hours, it was greatly increased at 24 and 48 hours. Therefore, in spite of remarkably increased liver injury, the animals are able to overcome injury and survive the potentiated liver toxicity (Kodavanti et al. 1992; Mehendale 1990, 1991, 1992). Some data suggest that the PB-induced P-450 isozyme(s) are more rapidly inactivated by carbon tetrachloride, and that PB pretreatment may alter the target lipids and/or the initiating metabolites involved in lipid peroxidation and diene conjugate formation (Moody 1992). DDT increased the sensitivity of rats to carbon tetrachloride poisoning (McLean and McLean 1966), and mice fed 100 ppm polybrominated biphenyls (PBBs) or 200 ppm polychlorinated biphenyls (PCBs) in their diet for 28 days experienced increased carbon tetrachloride hepatotoxicity (Kluwe et al. 1979). Potentiation of renal dysfunction was also found in the PBB-pretreated mice. All of these compounds are broad-spectrum mixed-function oxidase (MFO) inducers.

Concurrent treatment with methamphetamine at doses between 5 and 15 mg/kg increased hepatotoxicity in rats treated with carbon tetrachloride (Roberts et al. 1994). No potentiation occurred when metamphetamine was administered several hours before or after administration of carbon tetrachloride.

Low dietary doses (10 ppm) of the insecticides chlordecone or mirex (a structural analog of chlordecone) have been demonstrated to potentiate carbon tetrachloride hepatotoxicity. Chlordecone greatly enhanced the hepatotoxicity of carbon tetrachloride in rats, producing cholestasis as well as hepatocellular damage (Curtis et al. 1979). The investigators conclude that there is the likelihood of severe liver damage

resulting from interaction of carbon tetrachloride and chlordecone at exposure levels which may independently be nontoxic. Chlordecone has been reported not to potentiate the renal toxicity in rats (Kodavanti et al. 1992) or neurotoxicity in gerbils (Desaiah et al. 1991) of carbon tetrachloride, so its enhancing effects may be liver-specific. Chlordecone potentiation of carbon tetrachloride hepatotoxicity and lethality appears due to incapacitation of hepatocytes to regenerate and initiate the early phase of tissue repair. The authors also suggest that this is due to a precipitous depletion of cellular ATP that results from increased intracellular accumulation of Ca^{2+}, which in turn leads to a depletion of glycogen (Bell and Mehendale 1987; Mehendale 1990, 1991, 1992; Soni and Mehendale 1993). Mirex pretreatment of carbon tetrachloride-dosed rats was found not to produce cholestasis, but to produce a relatively modest increase in carbon tetrachloride hepatotoxicity (Bell and Mehendale 1985). Pretreatment of carbon tetrachloride-dosed rats with both mirex and chlordecone did not increase hepatotoxicity above that seen with chlordecone alone, indicating that chlordecone influenced susceptibility to carbon tetrachloride in a way independent of that of mirex. As proposed for phenobarbital, the mechanism underlying only limited and low-grade potentiation of carbon tetrachloride by mirex may involve a stimulation of hepatic regeneration and tissue repair that offsets cytochrome P-450 induction (Mehendale 1990, 1991, 1992). A single oral dose of chlordecone enhanced the oxidative metabolism of carbon tetrachloride in rats, but to a lesser degree than PB, which was in inverse relationship to these agents' effects on potentiation of the lethal and hepatotoxic effects of carbon tetrachloride (Mehendale and Klingensmith 1988). The investigators suggested the involvement as of yet unidentified factors, in addition to the modest enhancement of carbon tetrachloride metabolism, in chlordecone's unusually strong potentiating capacity. As discussed above, subsequent studies have suggested that chlordecone potentiates carbon tetrachloride-induced hepatotoxicity by depleting cellular energy stores, and consequently by inhibiting hepatocellular regeneration and liver tissue repair (e.g., Kodavanti et al. 1992; Mehendale 1991, 1992; Soni and Mehendale 1993).

Haloalkanes. Certain haloalkanes and haloalkane-containing mixtures have been demonstrated to potentiate carbon tetrachloride hepatotoxicity. Pretreatment of rats with trichloroethylene (TCE) enhanced carbon tetrachloride-induced hepatotoxicity, and a mixture of nontoxic doses of TCE and carbon tetrachloride elicited moderate to severe liver injury (Pessayre et al. 1982). The researchers believed that the interaction was mediated by TCE itself rather than its metabolites. TCE can also potentiate hepatic damage produced by low (10 ppm) concentrations of carbon tetrachloride in ethanol pretreated rats (Ikatsu and Nakajima 1992). Acetone was a more potent potentiator of carbon tetrachloride hepatotoxicity than was TCE, and acetone pretreatment also enhanced the hepatotoxic response of rats to a TCE-carbon tetrachloride mixture (Charbonneau et al. 1986). The potentiating

action of acetone may involve not only increased metabolic activation of TCE and/or carbon tetrachloride, but also possible alteration of the integrity of organelle membranes. Carbon tetrachloride-induced liver necrosis and lipid peroxidation in the rat have been reported to be potentiated by 1,2-dichloroethane in an interaction that does not involve depletion of reduced liver glutathione, and that is prevented by vitamin E (Aragno et al. 1992; Danni et al. 1992). Dichloromethane potentiated the hepatotoxicity of carbon tetrachloride in rats by increasing the covalent binding of carbon tetrachloride metabolites to hepatic microsomal lipids (Kim 1997). Several anesthetics (isoflurane, enflurane, halothane, and sevoflurane) enhanced the dechlorination of carbon tetrachloride by guinea pig microsomes by stimulating the reduction of cytochrome P-450 (Fujii 1996; Fujii et al. 1996).

Nicotine. Treatment of rats for 10 days with nicotine in drinking water increased liver histopathology (fatty change, necrosis, and dark-cell change) caused by an injection of carbon tetrachloride (Yuen et al. 1995). It was proposed that the increased hepatotoxicity might have resulted from a synergistic effect of the lipid peroxidation induced by both agents. Pregnant rats showed less severe effects than nonpregnant rats, possibly because of the differential hormonal status or differential expression of CYP-450 enzymes.

Carbon Disulfide and Other Alkyl Sulfides. Just as chemicals that serve to stimulate the metabolism of carbon tetrachloride lead to increased toxicity, chemicals that impair carbon tetrachloride metabolism lead to decreased toxicity. Rats dosed with carbon disulfide together with carbon tetrachloride displayed effects on the liver that resembled those due to carbon disulfide alone, rather than those caused by carbon tetrachloride alone (Seawright et al. 1980). This was judged to be due to destruction of the hepatic P-450 metabolizing system by carbon disulfide, such that activation of carbon tetrachloride was much reduced. Similar results have been reported in workers exposed to "80/20" (a mixture of carbon tetrachloride and carbon disulfide used to fumigate grain) (Peters et al. 1987). The neurological effects observed in these individuals resembled those caused by carbon disulfide alone, and there was no evidence of hepatotoxic effects characteristic of carbon tetrachloride exposure.

Other sulfides administered as pretreatments had different effects on carbon tetrachloride hepatotoxicity as measured by plasma ALT levels (Kim et al. 1996). The increase in plasma ALT levels induced by carbon tetrachloride was blocked by pretreatment with allyl sulfide or allyl disulfide and increased by pretreatment with propyl disulfide and butyl sulfide.

Dietary Status. Because carbon tetrachloride causes injury through oxidative pathways, depletion of cellular antioxidants such as glutathione, vitamin E, and methionine tend to increase the toxicity of carbon

tetrachloride. For example, feeding rats a diet low in vitamin E, selenium (a required cofactor for glutathione reductase), and methionine led to increased lipid peroxidation, while feeding a diet supplemented with one or more of these antioxidants tended to decrease lipid peroxidation (Hafeman and Hoekstra 1977) and oxidative liver damage (Parola et al. 1992). Similar results have been obtained by Taylor and Tappel (1976) and Sagai and Tappel (1978). In mice, retinoic acid or retinol inhibited the carbon tetrachloride-induced increase in serum alanine transaminase activity and liver histopathology, suggesting a protective effect of vitamin A in mice (Kohno et al. 1992; Rosengren et al. 1995). However, pretreatment with retinol increased hepatocyte injury in rats exposed to carbon tetrachloride (Badger et al. 1996; ElSisi et al. 1993a, 1993b).

Food deprivation has also been shown to have a substantial effect on carbon tetrachloride hepatotoxicity. A 24-hour fast significantly depressed hepatic glutathione (GSH) levels and enhanced carbon tetrachloride hepatotoxicity in rats (Harris and Anders 1980; Sato and Nakajima 1985), and promoted lipid peroxidation as measured by malondialdehyde formation (Ikatsu et al. 1991). A 1-day fast also increased hepatic injury as measured by increases in serum enzymes 2–2.5-fold compared to fed rats following 4-hour exposures at 500–2,500 ppm (Jaeger et al. 1982); the dietary status had no effect at 5,000 ppm. Diurnal decreases in hepatic GSH levels were found to coincide with periods of maximal susceptibility to carbon tetrachloride hepatotoxicity (Bruckner et al. 1984; Harris and Anders 1980). Even though the role of GSH in carbon tetrachloride cytotoxicity is poorly understood, it appears that more than GSH depletion is involved in fasting-induced enhancement of carbon tetrachloride hepatotoxicity. A 1-day fast stimulates the capacity of liver microsomes from male and female rats to metabolize carbon tetrachloride, although fasting did not produce a significant increase in hepatic microsomal protein or cytochrome P-450 levels (Nakajima and Sato 1979). Thus, short-term food deprivation may enhance the biotransformation of carbon tetrachloride to cytotoxic metabolites. Another factor in fasted animals was demonstrated in mice fasted for 24 hours that showed an 8-fold increase in hepatic triglycerides (steatosis) compared to untreated mice (Pentz and Strubelt 1983); it is likely that the increase in lipid content in the livers of fasted mice was responsible for their greater hepatic accumulation of injected carbon tetrachloride compared to fed mice. It should be recognized that food deprivation or consumption of a protein-free diet for several days diminishes MFO activity and makes rats more resistant to carbon tetrachloride (McLean and McLean 1966; Seawright and McLean 1967). Food restriction (25 or 50% lower caloric than control intake) for 30 days prior to administration of carbon tetrachloride and increased the carbon-tetrachloride-induced elevations in some serum enzymes in carbon-tetrachloride-treated rats. (Ramkumar et al. 2003; Seki et al. 2000). For example, the chemical-induced increase in serum AST was elevated 11-fold in female rats fed *ad libitum* but 27-fold in those on

a restricted diet (-25%) compared to controls. Food restriction (reduced by 25%) also increased the severity of lesions of the liver (hepatic cellular degeneration and fibrosis) and kidney (proximal tubular vacuolation and glomerular sclerosis) in treated rats compared to those fed *ad libitum* (Seki et al. 2000).

Metals. Pre-exposure to single doses of various metals (hexavalent chromium, mercuric chloride or silver) had no synergistic effect on lipid peroxidation in rats treated with carbon tetrachloride (Rungby and Ernst 1992). In mice fed a diet augmented with 3% carbonyl iron and intraperitoneally injected with carbon tetrachloride for 12 weeks, there were significant increases in parameters of hepatic injury (serum ALT, absolute and relative liver weight, severity of necrosis) compared to controls that were numerically larger than for groups treated with iron or carbon tetrachloride alone (Arezzini et al. 2003). Rats fed a low-copper diet were reported to be more sensitive to hepatic plasma membrane injury 24 hours following an intraperitoneal injection of carbon tetrachloride, possibly due to reduced Cu-Zn superoxide dismutase activities (DiSilvestro and Medeiros 1992). Rats fed a diet mildly deficient in zinc showed elevated levels of hepatocyte injury, as assessed by serum sorbitol dehydrogenase activity (DiSilvestro and Carlson 1994). In rats injected with lead nitrate and then carbon tetrachloride, hepatoxicity, as measured by serum ALT and AST, was lower than in rats injected with carbon tetrachloride alone (Calabrese et al. 1995); the authors attributed this effect to the ability of lead to inhibit cytochrome P-450.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to carbon tetrachloride than will most persons exposed to the same level of carbon tetrachloride in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of carbon tetrachloride, or compromised function of organs affected by carbon tetrachloride. Populations who are at greater risk due to their unusually high exposure to carbon tetrachloride are discussed in Section 6.7, Populations with Potentially High Exposures.

Section 3.9 discusses several types of compounds that can exacerbate the toxicity of carbon tetrachloride. Individuals exposed to these compounds may, therefore, be more sensitive to carbon tetrachloride exposure. As noted above, persons who are moderate to heavy drinkers are at significantly increased risk of liver and/or kidney injury following ingestion or inhalation of carbon tetrachloride (Manno et al. 1996). Occupational exposure to isopropanol has also been reported to markedly potentiate the hepatic or renal toxicity of carbon tetrachloride in men and women (Folland et al. 1976). This report and numerous

animal studies indicate that primary, secondary, and tertiary alcohols, as well as their ketone analogues, can substantially enhance the toxic potency of carbon tetrachloride. Substantial exposures to alcohols and ketones may occur in occupational settings or in certain instances in the use of household products containing these chemicals.

Drugs and other chemicals that significantly induce microsomal MFO activity can significantly increase the toxicity of carbon tetrachloride by enhancing its biotransformation to reactive, cytotoxic metabolites. A number of drugs such as phenobarbital, pentobarbital, and phenylbutazone are MFO inducers in animals and humans. Thus, individuals taking such medications may be at substantially greater risk of carbon tetrachloride toxicity. Other unusually susceptible individuals are those who have had significant exposures to insecticides such as DDT, chlordecone, or mirex, or to industrial chemicals such as PCBs or PBBs. All of these chemicals are potent MFO inducers and have been shown to markedly potentiate the hepatotoxicity of carbon tetrachloride in animals. Exposures to these chemicals can occur in industrial and agricultural settings, as well as in the general population via environmental media (i.e., contaminated water, food, air, and soil). Other widely used chemicals such as TCE have been found to enhance carbon tetrachloride toxicity in animals. Thus, persons with substantial exposure to TCE and other haloalkanes may be at greater risk of carbon tetrachloride toxicity.

Nutritional status can also influence the toxic potency of carbon tetrachloride. Animal studies have clearly demonstrated that brief fasting or consumption of diets low in antioxidants (vitamin E, selenium, methionine) can lead to increased carbon tetrachloride hepatotoxicity. The same may be true for humans, although this is not known for certain. Another aspect of nutritional status affecting carbon tetrachloride toxicity is hepatic energy status. Hepatic ATP levels might influence the ultimate outcome of toxicity (low levels may inhibit recovery mechanisms).

A variety of conditions may predispose certain segments of the population to carbon tetrachloride toxicity. Persons with alcoholic cirrhosis, or other liver diseases that have significantly diminished the functional reserve of the liver, have a reduced capacity to tolerate carbon tetrachloride-induced hepatotoxicity. The same is true for carbon tetrachloride-induced nephrotoxicity in people with significant renal dysfunction from other causes. Diabetics may be particularly susceptible to carbon tetrachloride poisoning, in light of animal studies that indicate elevated levels of ketone bodies induce the MFO system, which converts carbon tetrachloride to reactive, cytotoxic metabolites. Animal models for diabetes suggest different outcomes from exposure to carbon tetrachloride, depending on whether the disease is type 1 or type 2 (Sawant et al. 2004). Mice with type 1 diabetes, induced by intraperitoneal

injection with 200 mg/kg streptozotocin, showed no mortality after receiving a dose of carbon tetrachloride (1,594 mg/kg) that was lethal to half of non-diabetic mice (Shankar et al. 2003). Conversely, rats with type 2 diabetes, induced by administration of a high-fat diet and 45 mg/kg streptozotocin, showed 100% lethality at a dose of 3,188 mg/kg carbon tetrachloride that was not lethal to untreated controls or rats receiving a high-fat diet or streptozotocin alone (Sawant et al. 2004). The type 2 diabetic group had more severe hepatic necrosis between hours 12 and 36, greater depletion of hepatic glutathione at 6 hours, and a significant delay in the stimulation and progression of the S-phase of the cell division cycle compared to the other groups; CYP2E1 levels and rates of lipid peroxidation were not affected by type 2 diabetes in this animal model. Individuals with genetically-determined high MFO activity may be more susceptible to carbon tetrachloride toxicity, as may be persons with habits (e.g., smoking, consumption of smoked meats) that can produce increased MFO activity.

The organ-content of microsomal enzymes responsible for metabolizing carbon tetrachloride may change during different stages of the life cycle, indicating a potential for differing age-related susceptibilities following exposure. A number of studies on drug metabolism reported declines in hepatic activities of CYP2E1 and CYP3A3/4 in the elderly (>65 years) compared with earlier adult stages (as reviewed in Tanaka 1998). *In vitro* studies of human microsomes indicated that total immunoreactive CYP3A (the sum of CYP3A4 and CYP3A5) was significantly higher in livers from individuals aged 21–40 years compared to those aged 14–20 or 61–72 years (Patki et al. 2004). The observed lower rates of biotransformation of triazolam in the adolescent and elderly microsomal preparations was consistent with the reduced CYP3A content. In 18-month-old rats, no statistically significant reduction was observed in the mRNA or protein content for CYP2E1, but enzyme activity was reduced by 46% compared to 8-month-old rats (Wauthier et al. 2004). The reduced activity was attributed to inactivation of the enzyme over time by reactive metabolites. Reduced hepatic CYP3A content was noted in microsomes from 2-year-old CD-1 mice compared to 1-year-old mice (Warrington et al. 2000). Total CYP3A protein was also reduced in the liver of 25–26-month old F344 rats compared to younger animals, but was elevated by 11% in the kidney, largely because of a 50% upregulation of one isoform (Warrington et al. 2004). These results suggest that in F344 rats, ability of the kidney to generate reactive metabolites increases in the elderly.

Age-related reductions in antioxidant content would also tend to increase vulnerability to reactive metabolites of carbon tetrachloride in the elderly. Reductions in glutathione in old rats compared to younger animals have been noted in the liver and are associated with an age-related reduction in the transcription factor nuclear factor erythroid2-related factor (NrF2) that induces gammaglutamylcysteine

ligase, significantly involved with the synthesis of glutathione (Suh et al. 2004). In Wistar rats, both the liver and kidney of 22-month-old rats had significant decreases in glutathione and glutathione peroxidase, but increases in biomarkers of lipid peroxidation compared to 10-week-old rats (Martin et al. 2003). In F344 rats, glutathione peroxidase activity was significantly reduced in the kidney but not the liver of 24-month-old rats compared to 6-month-old rats (Tian et al. 1998); these results suggest a possible basis for the increasing sensitivity of the kidney in rats exposed to carbon tetrachloride by inhalation for 2 years (Japan Bioassay Research Center 1998). No study, however, has directly measured age-related differences in carbon tetrachloride metabolism in the kidney.

Genetic polymorphisms may confer differing susceptibilities to the effects of carbon tetrachloride exposure. In rat liver, two different forms of glutathione S-transferase 3-3 have been identified (Mayama et al. 2003). Hirosaki hairless rats are homozygous for the NC type gene (encoding Asn^{198}-Cys^{199}) and Sprague-Dawley rats are homozygous for the KS type gene (encoding Lys^{198}-Ser^{199}). When the two strains of rat were given an oral gavage dose of carbon tetrachloride, hepatic glutathione activity 0.5 hours later was reduced more significantly in NC rats compared to KS rats. Electrophoretic and chromatographic studies showed that the polymorphism affected the ability of the two kinds of enzyme to bind to heat shock protein 90-beta. The authors conclude that heat shock protein-beta protects the KS type of enzyme from inactivation by carbon tetrachloride.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to carbon tetrachloride. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to carbon tetrachloride. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide specific information about treatment following exposures to carbon tetrachloride:

Ellenhorn MJ. 1997. Ellenhorn’s medical toxicology: Diagnosis and treatment of human poisoning. 2nd ed. New York, NY: Elsevier, 1422-1429.

Leikin JB, Paloucek FP. 2002. Poisoning and toxicology handbook. 3rd ed. Hudson, OH: Lexi-Comp, Inc., 334.

Shih RD. 1998. Hydrocarbons. In: Goldfrank LR, Flomenbaum NE, Lewin NA, et al., eds Goldfrank's toxicologic emergencies. 6th ed. Stamford, CT: Appleton & Lange, 1383-1398.

3.11.1 Reducing Peak Absorption Following Exposure

Human exposure to carbon tetrachloride may occur by inhalation, ingestion, or dermal contact. Inhalation or oral exposure to carbon tetrachloride may cause hepatic, renal, and neurological effects. There is evidence, though limited, that dermal contact causes a similar pattern of effects.

If carbon tetrachloride has been inhaled, movement to fresh air is recommended. Humidified supplemental oxygen (100%) may be administered as required.

Ingestion of carbon tetrachloride should be considered a toxic emergency in which treatment should begin immediately. Treatment currently involves gastric emptying, either by gastric lavage (with a small bore nasogastric tube) or by induction of vomiting, preferably within minutes of exposure (Shih 1998). The patient needs to have a gag reflex and should not show signs of seizure, lethargy, or coma because of the risk of pneumonitis from pulmonary aspiration. In infants and young children, the induction of vomiting may induce severe fluid loss. Supportive therapy should be followed in all instances of treatment. A cathartic may be administered to speed fecal excretion (Ellenhorn 1997). Administration of activated charcoal is unlikely to be effective (Ellenhorn 1997). Animal studies revealed peak blood levels of carbon tetrachloride within 3–6 minutes after oral exposure when carbon tetrachloride was ingested undiluted or in aqueous vehicles by fasted rats (Kim et al. 1990a). Chemicals that induce P-450, such as ethanol and phenobarbital, should not be given. The administration of epinephrine is avoided, due to the possibility of inducing ventricular arrhythmias. In order to minimize absorption through the skin, all contaminated clothing should be removed and the skin should be washed with soap and water. In cases where the compound has been splashed into the eyes, irrigation with copious amounts of tepid water for 15 minutes has been recommended. Medical treatment is required if irritation, pain, swelling, lacrimation, or photophobia persist.

3.11.2 Reducing Body Burden

Hemodialysis may be employed in order to lower plasma carbon tetrachloride at the onset of renal failure (Ellenhorn 1997). Although this method is not very effective in removing lipophilic compounds from the blood, it is effective in controlling extracellular fluid composition if renal failure occurs (Ellenhorn 1997; EPA 1989b;). Because a substantial portion of absorbed carbon tetrachloride is exhaled within the first

hour, maintenance of a good tidal volume is recommended; hyperventilation may also be of value (Ellenhorn 1997). Administration of hyperbaric oxygen is an experimental treatment that is also available. Hyperbaric oxygen has been used in treating overdoses of carbon tetrachloride in humans (Larcan and Lorbet 1981; Truss and Killenberg 1982; Zearbaugh et al. 1988). Administration of hyperbaric oxygen following exposure to carbon tetrachloride improved survival from 31 to 96% in rats (Ellenhorn and Barceloux 1988). Hyperbaric oxygen has also been used in treating overdoses of carbon tetrachloride in humans and may correct regional tissue hypoxia and damage, as well as inhibit the P-450-dependent reductive dehalogenation of carbon tetrachloride to the metabolically active trichloromethyl radical in the liver. However, the effectiveness of this method has not been established in humans (Burkhart et al. 1991; Ellenhorn and Barceloux 1988).

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

Information is limited in humans regarding compounds that interfere with the mechanism of action of carbon tetrachloride. However, there is evidence that liver toxicity associated with exposure to carbon tetrachloride is mediated by reactive metabolites that bind to hepatocytes and initiate lipid peroxidation, thus resulting in loss of cell function. N-acetylcysteine has been suggested to bind the toxic metabolite phosgene and to serve as a precursor for the formation of glutathione (Ellenhorn and Barceloux 1988), and was protective against hepatotoxicity in carbon tetrachloride-exposed rats (Simko et al. 1992; Wong et al. 2003). Glutathione, a cellular antioxidant, tends to decrease lipid peroxidation due to carbon tetrachloride ingestion in rats (Arosio et al. 1997; Hafeman and Hoekstra 1977). Prior oral treatment with glutathione protected against hepatic necrosis, but did not modify lipid peroxidation or prevent covalent binding of carbon tetrachloride metabolites to hepatic microsomes in rats exposed intraperitoneally (Gorla et al. 1983). Agents that foster the maintenance of hepatic reduced glutathione levels have a similar protective effect against carbon tetrachloride: cysteine, a precursor to glutathione (De Ferreyra et al. 1974), taurine (Dincer et al. 2002; Vohra and Hui 2001; Waterfield et al. 1993), constituents of garlic oil such as diallyl trisulfide (Fukao et al. 2004), gamma-glutamylcysteinylethyl ester (Nishida et al. 1998), metformin, a dimethyl biguanide anti-hypoglycemic agent (Poon et al. 2003), and clofibrate (Manautou et al. 1998). Administration of 16,16-dimethyl prostaglandin E2 to block the accumulation of intracellular lipids has also been suggested (Haddad and Winchester 1990; Rush et al. 1986). Administration of fructose 1,6-diphosphate to rats has been shown to decrease carbon tetrachloride liver toxicity by increasing hepatocyte levels of ATP. The ATP thus generated is thought to promote hepatocellular regeneration and tissue repair (Rao and Mehendale 1989). Shertzer and Sainsbury (1991) reported that indole antioxidants 4b,5,9b,10-tetrahydroindeno[1,2-b]indole (THII) and 5,10-dihydroindeno-

[1,2-b]indole (DHII) inhibited carbon tetrachloride initiation of lipid peroxidation in rat liver microsomes, and protected against hepatotoxicity in rats when administered prior to carbon tetrachloride treatment. The authors suggested that these compounds may be suitable candidates for further development as potential chemoprotective and therapeutic agents for use in human disorders that involve free-radicals. Colchicine and trimethylcolchicinic acid, an analog that does not bind tubulin, prevented decreases in Ca^{2+}-ATP-ase activity, and reduced increases in gamma-glutamyl transpeptidase, alanine amino-transferase, and alkaline phosphatase in hepatocyte plasma membranes in rats treated with carbon tetrachloride (Cedillo et al. 1996; Martinez et al. 1995).

Oxygen supplementation improved ratios of ATP/ADP, inorganic phosphate/ATP, and lactate/pyruvate that had been altered in cirrhotic livers of rats previously treated with carbon tetrachloride (Harvey et al. 2000). These results were consistent with the hypothesis that hepatocyte damage in cirrhotic livers is exacerbated by a reduced oxygen supply and may partly explain the efficacy of hyperbaric oxygen therapy as described in Section 3.11.2).

Compounds that suppress the activity or expression of CYP2E1 have been shown to reduce the hepatic necrosis caused by the bioactivation of carbon tetrachloride. Pretreatment with 100–400 μmol/kg (subcutaneous) oleanolic acid, a triterpenoid compound, reduced heptatoxicity in rats and mice injected with carbon tetrachloride (Liu et al. 1998); the protective effect occurred 12–72 hours after pretreatment and was found to be unrelated to metallothionein levels. In mice, the protective effect of oleanolic acid was associated with inhibition of expression and activity of CYP2E1 (Jeong 1999). Another triterpenoid, alpha-hederin similarly reduced expression of CYP2E1 and hepatic injury in mice treated with carbon tetrachloride (Jeong and Park 1998). Methylenedioxybenzenes such as isosafrole, dihydrosafrole, and benzodioxole, administered 1 hour before carbon tetrachloride, prevented increases in plasma AST and ALT in mice (Zhao and O'Brien 1996). Isosafrole co-treatment also prevented the development of liver necrosis. Safrole was partially hepatoprotective, whereas piperonyl butoxide, eugenol, isoeugenol, sesamol, and curcumin were ineffective. Other similar compounds that prevented increases in plasma AST and ALT in rats included tetrahydro-5-methyl bis[1,3]benzdioxide [4,5-C: 5',6]-azecin-13 (5*H*)-one (protopine) (Janbaz et al. 1998) and 2-methylaminoethyl-4,4'-dimethoxy-5,6,5',6'-dimethylenedioxy-biphenyl-2-carboxylic acid-2'-carboxylate monohydrochloride (DBB-S) (Oh et al. 2000). A synthetic agent, 2-(allylthio)pyrazine, suppressed constitutive and inducible CYP2E1 expression and also blocked carbon tetrachloride-induced hepatotoxicity in mice (Kim et al. 1997); the compound also elevated hepatic GSH levels.

Tumor necrosis factor alpha (TNF-alpha) has been implicated in the process of hepatocellular injury following exposure to carbon tetrachloride. Co-treatment of rats with the soluble receptor to TNF-alpha reduced hepatocellular necrosis and the elevation in serum enzyme levels caused by carbon tetrachloride (Czaja et al. 1995). Mortality was 16% in the rats co-treated with the soluble receptor and 60% in rats co-treated with IgG.

A number of agents have been shown to reduce the severity of fibrosis induced in animals following intermediate-duration exposure to carbon tetrachloride. A weak but significant reduction in the area of carbon tetrachloride-induced hepatic fibrosis was measured by image analysis in rats co-treated with interferon alpha2a over a period of 9 weeks (Fort et al. 1998). There were concomitant reductions in several biochemical markers of fibrosis (hyaluronate, hydroxyproline, and the mRNAs for procollagen and fibronectin). In mice transgenic for the alpha(2)(I) collagen gene (COL1A2) promoter sequence and receiving a single intraperitoneal injection of carbon tetrachloride, interferon-alpha antagonized the transcription of COL1A2 that is stimulated by transforming growth factor-beta and the coactivator Smad3 (Inagaki et al. 2003); the progression of hepatic fibrosis was also prevented in interferon-treated mice. Administration of interferon-alpha2b also reduced the severity of fibrosis in the kidneys of rats subcutaneously injected with carbon tetrachloride over 7 weeks (Dogukan et al. 2003). Histopathology analysis revealed reductions in necrosis, dilatation and atrophy of renal tubules, hypercellularity of glomeruli, and obliteration of renal capillaries in rats co-treated with interferon compared to placebo-co-treated rats; the level of interstitial fibrosis was also reduced by interferon, although the difference was not statistically significant from the placebo co-treatment group. The kidneys of rats co-treated with interferon had more interstitial inflammation than the rats in the control group or in the placebo-co-treatment group. Pirfenidone (5 methyl-1-phenyl-2-(1H)-pyridone), an anti-fibrotic drug approved by the U.S. FDA for Phase II trials against pulmonary and renal fibrosis, reduced both the number of activated hepatic stellate cells and the severity of hepatic fibrosis when administered to rats with carbon tetrachloride-induced hepatic cirrhosis (Garcia et al. 2002); according to the authors, the anti-fibrotic effect of pirfenidone involves suppression of collagen gene transcription and possibly an inhibition of proline hydroxylase levels that would be expected to reduce the availability of hydroxyproline required for collagen synthesis.

Administration of liver growth factor to rats with hepatic cirrhosis following intraperitoneal injections of carbon tetrachloride for 10 weeks significantly improved the structure and function of the liver (Diaz-Gil et al. 1999). Significant decreases were observed in the levels of serum enzymes, the hepatic collagen content, and microscopic findings of fibrosis, necrosis, and inflammatory infiltration of the liver. In

addition, hepatic hemodynamic measures were improved in rats treated with liver growth factor compared to cirrhotic rats: reduced portal pressure and portosystemic shunting, reduced ascites, and increased mean arterial pressure and systemic vascular resistance. Implantation of rat fibroblasts genetically modified to express hepatic growth factor into the spleens of syngeneic rats significantly reduced hepatic injury (serum enzymes, histopathology) resulting from an intraperitoneal injection of carbon tetrachloride (Kaido et al. 1997). Gene therapy using an adenoviral vector bearing cDNA for a nonsecreted form of human urokinase plasminogen activator (Ad-ΔhuPA) reduced hepatic fibrosis in rats that became cirrhotic following treatment with carbon tetrachloride for 6–8 weeks (Salgado et al. 2000). The beneficial effect of enhanced uPA expression was partly attributed to its induction of hepatocyte growth factor.

Treatment of insulin-like growth factor-I (IGF-I) to rats during the last 3 weeks of exposure to carbon tetrachloride/phenobarbitol partially normalized the expression of 8 of 16 genes that were either up- or down-regulated in the cirrhotic liver (Mirpuri et al. 2002). Three of the genes affected by IGF-I are for protease inhibitors; restoration of the expression of these genes would be expected to protect against necrosis. IGF-I treatment also partially restored the expression of growth hormone receptor and the levels of global genomic DNA methylation, which are reduced during the development of cirrhosis (Mirpuri et al. 2002). Evaluation of hepatic effects following IGF-I administration to cirrhotic rats on the same protocol resulted in reductions in lipid peroxidation, fibrosis, and plasma AST and ALT, and increases in mitochondrial transmembrane potential (a measure of mitochondrial membrane integrity) (Castilla-Cortazar et al. 1997).

Several agents have been shown to ameliorate the effect of carbon tetrachloride on hepatic membranes. When co-administered with carbon tetrachloride, betaine, a mitochondrial metabolite of choline, reduced the extent of centrilobular steatosis and minimized the loss of hepatocyte organelle membranes (rough endoplasmic reticulum) in treated rats (Junnila et al. 2000); the effect was attributed to the enhancement of phospholipid synthesis necessary for maintaining the integrity of cell membranes. Hydroxychalcones, which have a 3,4-dihydroxycinnamoyl structure and inhibit lipoxygenases and cyclooxygenases, were potent inhibitors of lipid peroxidation in cultured rat hepatocytes (Sogawa et al. 1994). Polyenylphosphatidyl choline also reduced hepatic fibrosis induced by carbon tetrachloride in rats and accelerated the regression of existing fibrosis (Ma et al. 1996).

One effect of lipid injury following exposure to carbon tetrachloride is the release of hydrolytic enzymes such as calpain from lysosomes into the extracellular space where activation by calcium occurs (Limaye et al. 2003). As a result, cell necrosis progresses to neighboring cells, extending the hepatic lesion.

Administration of the calpain inhibitor *N*-CBZ-Val-Phe-methyl ester (CBZ) or the cell-impermeable inhibitor E64 1 hour after a toxic, nonlethal intraperitoneal dose of carbon tetrachloride protected against calpain-specific breakdown of alpha-fodrin, a cytoskeletal protein, and reduced the increase in serum ALT. Administration of CBZ 1 hour after a lethal dose (3 g/kg) increased survival from 25 to 75%. The calpain inhibitors have no effect on the metabolism of carbon tetrachloride by CYP2E1 or the generation of metabolites that bind to liver tissue.

As vitamin A (retinol) shows species-specific variations on carbon tetrachloride-related hepatotoxicity, it is not possible to predict whether it would be useful as a therapeutic agent in exposed humans. Pretreatment of male mice with vitamin A for 7 days prior to a single exposure to carbon tetrachloride reduced the elevations in plasma ALT levels as well as the extent of hepatic degeneration (Hooser et al. 1994). Some strain variations were evident in the protective effect of vitamin A, with no hepatocyte damage visible in C3H/He or athymic nude mice and only minimal hepatocyte damage visible near the central vein in Swiss-Webster or Balb/C mice. Conversely, pretreatment with vitamin A increased the hepatotoxicity (plasma ALT levels) of carbon tetrachloride 10-fold in male and female Sprague-Dawley rats, and male nude and Fischer-344 rats. The underlying basis for the species and strain differences is not known, but the possible involvement of Kupffer cells or polymorphonuclear neutrophils is under investigation. Inder et al. (1999) determined that the effect of vitamin A in Swiss-Webster mice does not involve alteration of the constitutive or inducible expression of CYP2E1.

Avid retention of Na^+ is a feature of liver cirrhosis. Icatibant (HOE 140), an antagonist to the bradykinin B_2 receptor, normalized Na^+ retention and reduced the hyperactivity of the renin-angiotensin-aldosterone system in rats that had become cirrhotic following treatment with carbon tetrachloride (Wirth et al. 1997).

Malnutrition is a common result of cirrhosis. Survival was improved in rats with carbon tetrachloride-induced cirrhosis by the dietary administration of branched-chain amino acids in addition to a casein diet (Kajiwara et al. 1998). Supplementation with branched-chain amino acids significantly preserved plasma albumin concentration and inhibited the occurrence of ascites and hyperammonemia without altering liver histopathology. The authors hypothesize that administration of branched-chain amino acids may suppress muscular protein catabolism and aid in detoxifying excess serum ammonia levels, which are characteristic of cirrhotic patients.

The protective effects of gadolinium a rare earth metal (lanthanide) and glycine against carbon tetrachloride injury operate via inactivation of Kupffer cells, which are hepatic macrophages (Rivera et al.

2001). When either compound was administered to rats with carbon tetrachloride-induced cirrhosis, the livers showed reductions in fibrosis, collagen protein, and transforming growth factor-beta-1 caused by carbon tetrachloride (Rivera et al. 2001). The inactivation of Kuppfer cells by glycine is suspected to be related to the inhibition of calcium signaling via glycine-gated chloride channels (Rivera et al. 2001). Gadolinium chloride also prevented liver injury and increased hepatocyte proliferation (as measured by immunostaining for the hepatocyte proliferating cell nuclear antigen) in rats when administered prior to treatment with carbon tetrachloride (Ishiyama et al. 1995). Gadolinium chloride inhibited CYP2E1 activity in cultured hepatocytes, reducing the loss of plasma membrane integrity caused by carbon tetrachloride (Badger et al. 1997).

Other substances that have been demonstrated to be protective against the toxic effects of carbon tetrachloride in animals include disulfiram (Brady et al. 1991), enprostil, an analog of prostaglandin E_2 (Bang et al. 1992), bosentan, and TAK-044, antagonists to the endothelin receptor (Hocher et al. 1995; Thirunavukkarasu et al. 2004), the xanthine oxidase inhibitor allopurinol (Dashti et al. 1992), the prolyl 4-hydroxylase inhibitors S 0885 and HOE 077 (Bickel et al. 1991), pyridoxol L,2-pyrrolidon-5 carboxylate (metadoxine) (Annoni et al. 1992), cyclosporine A (Farghali et al. 1996), the calcium antagonist nifedipine (Cutrin et al. 1992, 1994), alpha-tocopherol and derivatives (Hsiao et al. 2001; Liu et al. 1995), polyamines (Wu et al. 1997), adenosine (Hernandez-Munoz et al. 1992), various phenolic compounds (mostly flavinoids) (Adaramoye and Akinloye 2000; Cholbi et al. 1991; Pavanato et al. 2003), zinc (Camps et al. 1992), and chromium III (but not chromium IV) (Rungby and Ernst 1992; Tezuka et al. 1991a, 1991b). Supplementation with sodium tungstate for 7 weeks significantly reduced lipid peroxidation and necrosis produced by carbon tetrachloride in rats (Pawa and Ali 2004). A combination treatment with hyaluronic acid and chondroitin-4-sulfate (but not either agent alone) partly reduced the effects of carbon tetrachloride treatment (Camp et al. 2004); the therapy reduced hepatic necrosis and the increases in hepatic malondialdehyde, plasma TNF-alpha, and neutrophil-mediated myeloperoxidase and reversed the reduction in glutathione. Exercise has been shown to protect subsequently isolated rat hepatocyte from carbon tetrachloride cytotoxicity, probably by affecting cytochrome P-450-2E1 activity, and perhaps also by stimulating intracellular levels of free radical scavengers and antioxidants (Day and Weiner 1991). Food restriction (25 or 50% lower caloric than control intake) for 30 days prior to administration of carbon tetrachloride reduced the magnitude of blood lipid peroxidation and of increases in serum enzymes in carbon-tetrachloride treated rats (Ramkumar et al. 2003).

3.12 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of carbon tetrachloride is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of carbon tetrachloride.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

3.12.1 Existing Information on Health Effects of Carbon Tetrachloride

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to carbon tetrachloride are summarized in Figure 3-6. The purpose of this figure is to illustrate the existing information concerning the health effects of carbon tetrachloride. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a “data need”. A data need, as defined in ATSDR’s Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

As shown in Figure 3-6, there is a considerable body of data on the health effects of carbon tetrachloride in humans, especially following acute oral or inhalation exposures. Although many of the available reports lack quantitative information on exposure levels, the data are sufficient to derive approximate values for safe exposure levels. There is limited information on the effects of intermediate or chronic inhalation exposure in the workplace, but there are essentially no data on longer-term oral exposure of humans to carbon tetrachloride. Most toxicity studies have focused on the main systemic effects of

Figure 3-6. Existing Information on Health Effects of Carbon Tetrachloride

	Death	Acute	Intermediate	Chronic	Immunologic/Lymphoretic	Neurologic	Reproductive	Developmental	Genotoxic	Cancer
	Systemic									
Inhalation	●	●	●	●		●				●
Oral	●	●				●	●			
Dermal	●	●								

Human

	Death	Acute	Intermediate	Chronic	Immunologic/Lymphoretic	Neurologic	Reproductive	Developmental	Genotoxic	Cancer
	Systemic									
Inhalation	●	●	●	●		●	●	●	●	●
Oral	●	●	●	●	●	●	●	●	●	●
Dermal	●	●								

Animal

● Existing Studies

obvious clinical significance (hepatotoxicity, renal toxicity, central nervous system depression). There are data on the effects of carbon tetrachloride on the immune system, but there are no reports that establish whether or not developmental, reproductive, genotoxic, or carcinogenic effects occur in humans exposed to carbon tetrachloride.

The toxicity of carbon tetrachloride has been extensively investigated in animals, both by oral and inhalation exposure. While the majority of existing studies in animals have focused on systemic toxicity (hepatic and renal injury), several studies have examined the neurologic, developmental, and reproductive effects of carbon tetrachloride. Effects of carbon tetrachloride on the immune system have been studied following oral, but not after inhalation or dermal exposure. The carcinogenicity of carbon tetrachloride has been studied in animals following inhalation or oral exposure.

3.12.2 Identification of Data Needs

Despite the phase-out of carbon tetrachloride manufacture and use in many areas of the world, its environmental persistence may support the continued practical relevance of many of the data needs identified below.

Acute-Duration Exposure. A large number of studies are available regarding the effects of single exposures to carbon tetrachloride, both in animals and humans. Available data indicate that the central nervous system, liver, and kidneys are primary target organs for carbon tetrachloride. Many of these studies involved exposure to only one dose level (usually high enough to cause clear effects), and the minimum dose needed to produce the characteristic effects of carbon tetrachloride toxicity is not defined with certainty. Although human studies exist, data were not suitable for derivation of an acute inhalation MRL. An acute inhalation MRL was not derived because calculations based on the most suitable data (exposure of rats at 10 ppm, 7 hours/day for 13 exposures over 17 days in the study by Adams et al. 1952), would result in a value (0.02 ppm) lower than the intermediate-duration inhalation MRL. Another assay by Adams et al. (1952) in which rats were exposed for 7 hours on a single day used too small group sizes. The intermediate-duration inhalation MRL of 0.03 ppm is expected to be protective for acute-duration inhalation exposures. An acute-duration oral MRL of 0.02 mg/kg/day was derived based on a LOAEL of 5 mg/kg/day in rats exposed on 10 consecutive days (Smialowicz et al. 1991). Further studies in animals, involving a range of exposure levels and employing sensitive histological and biochemical measurements of injury to liver and kidney, would be helpful in defining the thresholds for acute hepatic

and renal toxicity. Studies on the time-course of changes in the most sensitive parameters would be valuable. Most studies are conducted 18–24 hours after exposure. Because carbon tetrachloride is so rapidly absorbed and distributed to target tissues, significant biochemical and histological changes may occur within minutes. These changes may not be evident 18–24 hours later (e.g., Mehendale 1991, 1992). Data for all exposure routes would be valuable, but further information on inhalation and dermal dose-response relationships would be particularly helpful. In addition, dose-response studies of the effects of acute exposures on other tissues and systems (e.g., nervous, immune, reproductive, developmental) would be useful in determining whether other tissues are injured, especially at doses near the thresholds for injury to the liver and kidney. Furthermore, for purposes of enhancing toxicity and risk assessments related to carbon tetrachloride exposure, dose-response studies in species other than rats and gerbils on induced compensatory mechanisms (e.g., hepatocellular regeneration and tissue repair; see, for example, Calabrese et al. 1993; Kodavanti et al. 1992; Mehendale 1990, 1991, 1992; Rao and Mehendale 1991, 1993) might also prove useful.

Intermediate-Duration Exposure. The effects of repeated exposure to carbon tetrachloride have been investigated in a relatively small number of studies. Similar target organs were reported as those for acute-duration exposure. An intermediate-duration inhalation MRL of 0.03 ppm was derived for liver effects based on a NOAEL of 5 ppm in rats (Adams et al. 1952). An intermediate-duration oral MRL of 0.007 mg/kg/day was derived based on a NOAEL of 1 mg/kg/day in animals (Bruckner et al. 1986). There are a number of areas where further studies would be useful. Most oral studies of carbon tetrachloride toxicity in animals have involved administration of carbon tetrachloride by gavage in corn oil (Condie et al. 1986; Kim et al. 1990b). Since a bolus dose in oil may produce effects somewhat different from those following intermittent exposure in water (e.g., greater hepatotoxicity when administered in oil, Condie et al. 1986), studies involving exposure in drinking water would be valuable, especially since this is a likely exposure pathway for residents using private wells near hazardous waste sites. More information on the adverse effect levels and mechanism of toxicity in tissues other than the liver (e.g., the kidney and nervous system) would be useful; since many oral exposure studies examined only the liver, adverse effect levels for other organ systems are not well characterized.

Chronic-Duration Exposure and Cancer. No definitive studies were located in humans on the noncarcinogenic effects of carbon tetrachloride after chronic-duration exposure. An occupational study by Tomenson et al. (1995) evaluated liver function, as indicated by the levels of hepatic enzymes in serum, in a cross-sectional study of individuals occupationally exposed to carbon tetrachloride. Although the exposed workers were categorized by their length of time on the job (<1, 1–5, and >5 years), this

information was not included in the exposure-response analysis, so the effect of exposure duration is uncertain. A chronic inhalation MRL of 0.03 ppm was derived based on a NOAEL of 5 ppm and a LOAEL of 25 ppm for hepatic effects in rats exposed 6 hours/day, 5 days/week for 2 years (Japan Bioassay Research Center 1998; Nagano et al. 1998). Increased incidence of chronic progressive nephropathy in rats occurred at the same NOAEL and LOAEL; significantly elevated proteinuria (4+) was observed at a LOAEL of 5 ppm, but was not used for derivation of the MRL because intrinsic levels in controls were so high (>90% scoring 3+ or 4+). At the doses used in chronic oral bioassays, increases in the incidence and severity of non-neoplastic hepatic lesions in rats, the incidence of hepatic cancer in mice, and mortality in both sexes were too high to provide a basis for a chronic oral MRL. A study is needed to determine no-effect levels for hepatotoxicity following chronic-duration oral dosing with carbon tetrachloride.

The carcinogenicity of carbon tetrachloride was evaluated in rats and mice exposed intermittently by inhalation for 2 years (Japan Bioassay Research Center 1998; Nagano et al. 1998). These assays provided sufficient data for hepatic carcinogenicity in both sexes, and some evidence for a threshold effect in both species. The adrenal gland in mice was the only other tissue that had an increased tumor incidence. There is ample evidence that oral (Andervont 1958; Della Porta et al. 1961; Edwards 1941; Edwards et al. 1942; Eschenbrenner and Miller 1944, 1946; NCI 1976a) and parenteral (Della Porta et al. 1961; Reuber and Glover 1967b, 1970) exposure to carbon tetrachloride can lead to increased tumor frequency in animals, but there is currently no dose-response information for carcinogencity at more relevant oral exposure levels. Results of oral gavage studies could be used to plan dose levels for studies in which the chemical is administered in drinking water, as more relevant to actual human exposure scenarios . Current oral data was derived from animals dosed by corn oil bolus gavage, a method of dosing that does not reflect human exposure calculations, and may overestimate the risk as has been suggested by studies of other chlorinated methane and ethane compounds (Jorgenson et al. 1985; Kleming et al. 1986). While the carcinogenic risks of chronic dermal exposure have not been studied, chronic dermal exposure to carbon tetrachloride is not likely for most individuals.

Genotoxicity. Although it is evident that carbon tetrachloride exposure can increase the incidence of tumors in animals, it is not certain whether carbon tetrachloride is acting via a genotoxic mechanism, as a promoter, or some other process. Nearly all studies to date have failed to demonstrate any genotoxicity of unmetabolized carbon tetrachloride, although reactive metabolites and lipid peroxidation products are genotoxic, forming adducts with DNA (Castro et al. 1989; Chaudhary et al. 1994; Chung et al. 2000; Wacker et al. 2001). Since it is believed that carbon tetrachloride toxicity is mediated at least in part

through highly reactive and short-lived metabolites, further studies should focus particular attention on the issue of metabolic activation (especially anaerobic, reductive reactions), with *in vivo* or intact eukaryotic cell systems capable of activation *in situ* being preferred over systems relying on exogenous activation.

Reproductive Toxicity. The effects of carbon tetrachloride on reproduction have not been well investigated. Inhalation of carbon tetrachloride caused testicular degeneration (Adams et al. 1952) and reduced fertility (Smyth et al. 1936) in rats, but at doses higher than the adverse effect level for hepatotoxicity. Oral exposure to carbon tetrachloride did not adversely affect reproduction in one study in rats, but there is uncertainty as to the actual doses administered in this study (Alumot et al. 1976). Additional studies in animals using modern techniques and protocols would be useful to evaluate dose-response relationships for functional reproductive parameters in males and females.

Developmental Toxicity. Epidemiological studies have been published on the developmental effects of carbon tetrachloride in humans (Bove et al. 1992a, 1992b, 1995; Croen et al. 1997). Limited data suggest that carbon tetrachloride has a low potential for developmental toxicity in animals. Fetal size was reduced and viability and lactation indices were decreased following inhalation exposures at or above 250 ppm (Gilman 1971; Schwetz et al. 1974). Fetotoxicity and teratogenicity were not seen in offspring coming to term, but total resorption of fetuses occurred in pregnant rats following oral exposure (Narotsky et al. 1997a, 1997b; Wilson 1954). Metabolic studies suggest that the fetuses of several rodent species, including the rat, lack the enzymes needed for activation of carbon tetrachloride, and that this may explain the low developmental toxicity. However, this phenomenon may not apply to humans, where some drug metabolizing activity takes place *in utero*, especially in the fetal brain (Brezinski et al. 1999). It would be useful to find nonrodent animal models, possibly primates, in which the MFO system also develops *in utero*, and use these to study the developmental toxicity of carbon tetrachloride. Studies are needed to evaluate the possible neurological or neurobehavioral effects of gestational exposure to carbon tetrachloride; parallel groups to evaluate the effect of maternal exposure to ethanol, which induces CYP2E1 would also be relevant to humans.

Immunotoxicity. There are a number of reports that parenteral exposure of animals to carbon tetrachloride can affect the immune system (Kaminski et al. 1989, 1990; Tajima et al. 1985). The effects of carbon tetrachloride on the immune system have been investigated following oral dosing (Smialowicz et al. 1991), but no immunotoxicity was observed at doses much higher than those causing hepatic toxicity. Intermediate- and chronic-duration inhalation bioassays in rodents reported increased spleen

weights, but, as indicated by hemosiderin deposition, this effect appears to be secondary to erythrocyte toxicity and not an immunological effect *per se* (Japan Bioassay Research Center 1998). Although some older reports suggested that dermal exposure to carbon tetrachloride may result in a hypersensitization reaction (McGuire 1932; Taylor 1925), additional dermal exposure studies do not appear to be of high priority.

Neurotoxicity. Available data make it clear that the central nervous system is a target organ for carbon tetrachloride, with the most obvious acute effects being central nervous system depression (Cohen 1957; Stevens and Forster 1953; Stewart et al. 1963). Although our understanding of this important aspect of carbon tetrachloride toxicity might benefit from further study of animals and accidentally exposed humans, of greater concern are the scattered reports that carbon tetrachloride exposure causes focal injury and degeneration of peripheral neurons. Additional studies by inhalation and oral routes would be helpful in defining the dose-response dependency of nerve cell injury, and in determining whether these effects are primary or are secondary to effects on the liver or kidneys.

Epidemiological and Human Dosimetry Studies. Several epidemiological studies have been conducted on the health effects of intermittent workplace exposure to carbon tetrachloride, primarily evaluating the effects on the central nervous system (Elkins 1942; Heimann and Ford 1941; Kazantzis and Bomford 1960), hepatic (Barnes and Jones 1967; Smyth et al. 1936; Tomenson et al. 1995), and renal (Barnes and Jones 1967) function in relatively small groups of workers. Cancer epidemiological studies have been conducted on significantly larger subject groups (Blair et al. 1998; Bond et al. 1986; Cantor et al 1985; Checkoway et al. 1984; Dumas et al. 2000; Heineman et al. 1994; Kernan et al. 1999; Wilcosky et al. 1984). Epidemiological studies evaluated developmental effects (Bove et al. 1992a, 1992b, 1995; Croen et al. 1997) in populations exposed to carbon tetrachloride in drinking water, which is a route of exposure that may be of concern near hazardous waste sites. A common problem in epidemiological studies is the acquisition of reliable dosimetry data on the exposed populations. For this reason, efforts to improve estimates of past exposures and to define more accurately current exposure levels to carbon tetrachloride would be valuable.

Biomarkers of Exposure and Effect.

Exposure. The presence of carbon tetrachloride in expired air is the most commonly used biomarker of exposure. The rate of excretion in humans appears to be biphasic, with an initial elimination half-life of less than 1 hour, and a second phase of about 30–40 hours. The compound can be detected in expired air

within hours to weeks after exposure. Research on additional biomarkers of exposure would be of value, perhaps in areas such as detection of DNA adducts.

Effect. There are a number of clinical and biochemical tests available that can detect early signs of hepatic and renal injury in humans. However, these tests are not specific for carbon tetrachloride-induced effects. For this reason, studies to identify and measure effects more diagnostic of carbon tetrachloride-specific injury would be helpful. Also, improvements in the sensitivity of these tests, such as accomplished by Ikemoto et al. (2001), would be valuable in evaluating the health status of individuals who have been exposed to low levels of carbon tetrachloride.

Absorption, Distribution, Metabolism, and Excretion. There is relatively little quantitative information on the systemic absorption of inhaled carbon tetrachloride in animals and humans, with estimates ranging from 30 to 60% (Lehmann and Schmidt-Kehl 1936; McCollister et al. 1951). Sanzgiri et al. (1995, 1997) have compared uptake, distribution, and elimination of carbon tetrachloride administered to rats over 2 hours by inhalation or gastric infusion or as a single bolus by gavage and correlated the results with the severity of hepatic injury. This study provides information pertinent to a route-to-route extrapolation.

Although dermal absorption of carbon tetrachloride is relatively modest compared to absorption by the oral or inhalation routes, it would be helpful to quantify the rate and extent of percutaneous absorption of carbon tetrachloride from water. This information would be useful in determining the contribution of dermal exposure to the total dose received by persons using carbon tetrachloride-contaminated drinking water for bathing or showering, or to those who contact carbon tetrachloride-contaminated water near chemical waste sites.

Animal studies reveal that carbon tetrachloride is distributed to tissues according to their rate of blood perfusion and lipid content. Adipose tissue accumulates much higher concentrations of carbon tetrachloride than other tissues, due to the high oil:water partition coefficient of carbon tetrachloride. The animal tissue distribution data are limited, in that carbon tetrachloride levels in tissues in rats have been determined at only a few time-points after a single, high oral dose (Marchand et al. 1970; Teschke et al. 1983). Paustenbach et al. (1986a, 1986b) have measured ^{14}C-carbon tetrachloride levels in tissues of rats at just one time-point following repeated inhalation exposure regimens.

Although numerous studies have investigated the metabolism of carbon tetrachloride in the liver, there is little information about rates of metabolism or relevant CYP-450 proteins in the kidney, to which a substantial proportion of absorbed carbon tetrachloride is distributed under gradual exposures such as inhalation or gastric infusion (Sanzgiri et al. 1997). Investigation of these processes would provide a basis for understanding the increased prominence of renal toxicity in rats under intermediate- and chronic-duration inhalation exposures (Japan Bioassay Research Center 1998).

Comparative Toxicokinetics. Metabolic pathways and mechanisms of hepatotoxicity of carbon tetrachloride have been the subject of many studies in intact animals and *in vitro*, and are therefore better understood than for many other chemicals. However, there are apparently little data on metabolism of carbon tetrachloride in humans. It would be valuable to conduct *in vitro* experiments with human liver samples and hepatocytes to determine whether metabolic pathways and toxic metabolites are similar to those found in animals. It would also be beneficial to identify an animal model in which MFO systems develop in utero as they do in the human fetus.

PBPK models have been developed for a number of drugs and chemicals, in order to better understand and simulate the dynamics of those compounds in the body. Advances made to date indicate that valid PBPK models can accurately predict the concentration of chemicals over time in the blood and specific tissues. Blood and tissue concentration versus time profiles, as well as excretion patterns from animals have been used to validate and adjust PBPK models for carbon tetrachloride (Gallo et al. 1993; Paustenbach et al. 1988). Addition of parameter values for humans has been used to scale-up the PBPK model to predict target tissue uptake, metabolism, and elimination of carbon tetrachloride in humans (Thrall et al. 2000). One limitation of using current models for deriving human equivalent concentrations from animal data for the purposes of MRL derivation is that there is insufficient information on the rates of metabolism for carbon tetrachloride for the general population. Thrall et al. (2000) estimated the *in vivo* metabolic rate (V_{max}) in humans from animal *in vivo* rates and *in vitro* results for animal and human hepatic microsomes. However, the *in vivo* rate derived for humans was based on microsomes pooled from only three individuals (Zangar et al. 2000) and without additional studies, it is not known whether the rate is typical of the general population. Additional studies to characterize the variability of metabolic rates in humans would help to reduce the uncertainty associated with the application of the PBPK model. Quantitative relationships between carbon tetrachloride levels in target organs and organ damage in animals could be used to establish toxicodynamic models. Accurate prediction of ultimate toxicological outcomes will likely also have to account for base-line and inducible levels of compensatory repair

mechanisms. Combined PBPK-toxicodynamic models might then be scaled up and used to predict target organ concentrations and toxicity of carbon tetrachloride in man.

Methods for Reducing Toxic Effects. The usefulness of methods and treatments for reducing peak absorption and reducing the body burden of carbon tetrachloride is rather limited due to the chemical's rapid rates of absorption and tissue disposition. On the other hand, investigations of antidotal therapy based on the mechanism of action have been limited to a few studies involving the administration of compounds to reduce free radical injury. Additional studies would be useful to better establish the effectiveness of both acute and prolonged antidotal therapy, since carbon tetrachloride is persistent in the body.

Children's Susceptibility. Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection above.

The difference between the toxicity of carbon tetrachloride in children and adults is likely to be dependent on the relative expression of microsomal enzymes, such as CYP2E1. Viera et al. (1996) determined that hepatic levels of CYP2E1 in children reach adult levels sometime between the ages of 1 and 10. Additional studies are needed to obtain a precise chronology of the increase. Furthermore, additional studies are needed to clarify fetal expression of CYP2E1 to determine the sensitivity of different fetal tissues and the placenta during gestation.

Child health data needs relating to exposure are discussed in Section 6.8.1, Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

Numerous current publications on carbon tetrachloride have addressed the efficacy of various agents for reducing or eliminating the toxic effects of exposure; these are mentioned in Section 3.11.3. Additional research programs are focusing on potential therapeutic agents, interacting factors, or mechanisms of toxicity following exposure to carbon tetrachloride. These studies are listed in Table 3-8.

Table 3-8. Ongoing Studies on the Health Effects of Carbon Tetrachloride

Investigator	Affiliation	Research description	Sponsor
Alpini GD	Texas A&M University	Role of protein kinase C in regulating biliary damage from carbon tetrachloride.	NIH-NIDDKD
Anania FA	University of Maryland	Role of leptin in liver fibrogenesis.	NIH-NIDDKD
Bacon BR	St. Louis University	Signaling pathways in hepatic fibrogenesis.	NIH-NIDDKD
Carrasquillo JA	NIH Clinical Center	Development and use of Tc-99m, a radiopharmaceutical as a hepatobiliary agent to visualize effects of carbon tetrachloride *in vivo*.	NIH
Devilliers WJ	University of Kentucky	Role of CD36, a class B scavenger receptor, on activation of hepatic stellate cells and hepatic fibrosis.	NIH-NIAAA
Dranoff JA	DVA, Medical Center, West Haven, Connecticut	Effects of liver injury on nucleotide receptors.	DVA
Gandhi CR	University of Pittsburgh	Endothelin receptor involvement in development of hepatic cirrhosis following exposure to carbon tetrachloride.	NIH- NIDDKD
Kavanagh TJ	University of Washington	Role of glutamate-cysteine ligase in antioxidant defense against hepatic injury.	NIH-NIEHS
Lambris JD	University of Pennsylvania	Role of complement in liver regeneration	NIH-NIDDKD
Lu SC	University of Southern California	Support research on cultured hepatic parenchymal and non-parenchymal cells as models of hepatic injury from carbon tetrachloride and other hepatotoxins.	NIH-NIDDKD
Manautou JE	University of Connecticut	Evaluation of upregulation of canalicular ATP-dependent efflux pump (cMRP or C MOAT) for organic ions and hepatocellular regeneration after carbon tetrachloride exposure.	NIH-NIEHS
Mason R	NIEHS	Role of nitric oxide in metabolism of toxic chemicals.	NIH-NIEHS
Mason R	NIEHS	Biomarkers of oxidative stress in carbon tetrachloride -induced hepatotoxicity.	NIEHS
Mehendale HM	University of Louisiana at Monroe	Investigate factors associated with resiliency in aged rats to hepatotoxicity from chlordecone and carbon tetrachloride.	NIH-NIA

Table 3-8. Ongoing Studies on the Health Effects of Carbon Tetrachloride

Petersen BE	University of Florida	Study subpopulations of oval cells derived from bone marrow for therapeutic purposes following carbon tetrachloride hepatotoxicity.	NIH-NIDDKD
Song BJ	NIAAA	Regulation and role of CYP2E1 in mechanism of hepatic injury.	NIH-NIAAA
Tyner AL	University Illinois at Chicago	Role of cyclin kinase inhibitors p21 and p27 in carbon tetrachloride -induced hepatotoxicity.	NIH-NIDDKD

Sources: CRISP 2004; FEDRIP 2004

CRISP = Computer Retrieval of Information on Scientific Projects; DVA = Department of Veterans Affairs; FEDRIP = Federal Research in Progress database; NIAAA = National Institute on Alcohol Abuse and Alcoholism; NIDDKD = National Institute of Diabetes and Digestive and Kidney Diseases; NIEHS = National Institute of Environmental Health Services; NIH = National Institute of Health

4. CHEMICAL AND PHYSICAL INFORMATION

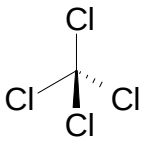
4.1 CHEMICAL IDENTITY

Information regarding the chemical identity of carbon tetrachloride is located in Table 4-1.

4.2 PHYSICAL AND CHEMICAL PROPERTIES

Information regarding the physical and chemical properties of carbon tetrachloride is located in Table 4-2.

Table 4-1. Chemical Identity of Carbon Tetrachloride

Characteristic	Information	Reference
Chemical name	Carbon tetrachloride	IARC 1979
Synonym(s)	Carbona; carbon chloride; carbon tet; methane tetrachloride; perchloromethane; tetrachloromethane; benzinoform	HSDB 2004
Registered trade name(s)	Benzinoform; Fasciolin; Flukoids; Freon 10; Halon 104; Tetraform; Tetrasol	IARC 1979
Chemical formula	CCl_4	IARC 1979
Chemical structure		IARC 1979
Identification numbers:		
CAS registry	56-23-5	NLM 1988
NIOSH RTECS	FG4900000	HSDB 2004
EPA hazardous waste	U211; D019	HSDB 2004
OHM/TADS	7216634	HSDB 2004
DOT/UN/NA/IMCO shipping	UN1846; IMCO 6.1	HSDB 2004
HSDB	53	HSDB 2004
NCI	No data	

CAS = Chemical Abstracts Service; DOT/UN/NA/IMCO = Department of Transportation/United Nations/North America/International Maritime Dangerous Goods Code; EPA = Environmental Protection Agency; HSDB = Hazardous Substances Data Bank; NCI = National Cancer Institute; NIOSH = National Institute for Occupational Safety and Health; OHM/TADS = Oil and Hazardous Materials/Technical Assistance Data System; RTECS = Registry of Toxic Effects of Chemical Substances

Table 4-2. Physical and Chemical Properties of Carbon Tetrachloride

Property	Information	Reference
Molecular weight	153.82	Lide 1993
Color	Colorless	Verschueren 1983
Physical state	Liquid	Verschueren 1983
Melting point	-23 °C	Lide 1992
Boiling point	76.5 °C	Lide 1992
Density	1.594 g/mL	Lide 1992
Odor	Aromatic, sweet	HSDB 2004
Odor threshold:		
Water	0.52 mg/L	IRIS 2004
Air	10–71,000 mg/m^3	Verschueren 1983
	96 ppm (600 mg/m^3)	Amoore and Hautala 1983
	60–1,500 mg/m^3	Ruth 1986
Solubility:		
Water at 20 °C	800 mg/L	Verschueren 1983
Organic solvent(s)	Miscible	HSDB 2004
Partition coefficients:		
Log K_{ow}	2.64	EPA 1984
Log K_{oc}	2.04	Kenaga 1980
Vapor pressure at 20 °C	90 mmHg	Verschueren 1983
Henry's law constant:		
at 25 °C	2.94x10^{-2} atm-m^3/mol	Yaws et al. 1991
at 24.8 °C	3.04x10^{-2} atm-m^3/mol	HSDB 2004
at 20 °C	2.04x10^{-2} atm-m^3/mol	Tse et al. 1992
at 30 °C	3.37x10^{-2} atm-m^3/mol	Tse et al. 1992
Autoignition temperature	Nonflammable	HSDB 2004
Flashpoint	Nonflammable	HSDB 2004
Flammability limits	Nonflammable	HSDB 2004
Conversion factors		
ppm (v/v) to mg/m^3 in air (25 °C)	1 ppm=6.39 mg/m^3	HSDB 2004
mg/m^3 to ppm (v/v) in air (25 °C)	1 mg/m^3=0.16 ppm	Verschueren 1983
Explosive limits	No data	

5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

5.1 PRODUCTION

Carbon tetrachloride is produced by exhaustive chlorination of a variety of low molecular weight hydrocarbons such as carbon disulfide, methane, ethane, propane, and ethylene dichloride (HSDB 2004). It is also produced by thermal chlorination of methyl chloride (HSDB 2004). Carbon tetrachloride is a feedstock for chlorofluorocarbon gases, such as dichlorodifluoromethane (F-12) and trichlorofluoromethane (F-11), which were used as aerosol propellants in the 1950s and 1960s (Holbrook 1991). Following this, the growth rate for the production of carbon tetrachloride averaged 10.7% per year from 1960 to 1970 (Holbrook 1991). This rate slowed to 7.2% per year from 1970 to 1974, when the production of this chemical was at its peak, as other forms of propellants became commercially available (Anonymous 1981; Holbrook 1991). The FDA banned the sale of carbon tetrachloride in any product used in the home and the EPA regulated the use of chlorofluorocarbon gases as aerosols or propellants. Since then, production of carbon tetrachloride has declined at approximately 8% a year from 1974 to 1994 (Anonymous 1995; Holbrook 1991). Carbon tetrachloride is currently manufactured in the United States by Vulcan Materials Company at two plants: Geismar, Louisiana and Wichita, Kansas, with a combined 130 million pound capacity (HSDB 2004; SRI 2004). It should be noted, however, that these capacities are flexible, since other chlorinated solvents are made using the same equipment (SRI 2004).

This recent decline in production is due to the adoption of an international agreement (the Montreal Protocol) to reduce environmental concentrations of ozone-depleting chemicals (including carbon tetrachloride), and to the provisions of Title VI of the Clean Air Act Amendments of 1990 addressing these chemicals. The regulation called for reduction to 15% of 1989 production levels by 1995 and a complete phase-out of carbon tetrachloride production for nonfeedstock uses by 2000. The EPA allocated a baseline production allowance of about 138 million pounds (63,000 metric tons) of carbon tetrachloride, apportioned among the eight U.S. companies producing the chemical in 1989 (EPA 1991a).

5.2 IMPORT/EXPORT

The trend in recent years has shown a drop off in both imports and exports for carbon tetrachloride. (Anonymous 1983, 1995). Current import or export quantities show that for the year 2002, the United

States exported 11,880,074 kg (1,880 metric tons), and for 2003 through April, the United States exported 3,714,817 kg (3,715 metric tons) (USITC 2003). Imports for both years were reported at <50 kg. Table 5-1 summarizes information on U.S. companies that reported the production, import, or use of carbon tetrachloride for the Toxics Release Inventory (TRI) in 2002 (TRI02 2004). The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list.

5.3 USE

The major use of carbon tetrachloride has historically been for the production of chlorofluorocarbons, such as dichlorodifluoromethane (F-12) and trichlorofluoromethane (F-11), which are used primarily as refrigerants as mentioned in section 5.1 (Holbrook 1991; HSDB 2004). Carbon tetrachloride found a variety of other uses in the past in industry, in medicine, and in the home. In the early part of this century, carbon tetrachloride was taken by mouth as a treatment for intestinal worms (Hall 1921), and it was also used briefly as an anesthetic (Hardin 1954). Because carbon tetrachloride is a solvent, it has been widely used as a cleaning fluid in the home and as a degreaser in industry. Because it is nonflammable, it was also used in fire extinguishers. Until recently, it was used as solvent in some household products and as a fumigant to kill insects in grain. It has been estimated that 28 million pounds of carbon tetrachloride were used as a fumigant in 1978 (Daft 1991). Because of the toxicity of carbon tetrachloride, consumer and fumigant uses have been discontinued, and only industrial uses remain (HSDB 2004).

Since production of carbon tetrachloride for most remaining uses has been phased-out due to Clean Air Act legislation (see Section 5.1), the chemical is only available for those uses for which no effective substitute has been found.

5.4 DISPOSAL

EPA classifies carbon tetrachloride and waste containing carbon tetrachloride as hazardous wastes. Generators of waste containing this contaminant must conform to EPA regulations for treatment, storage, and disposal (see Chapter 8). Rotary kiln or fluidized bed incineration methods are acceptable disposal methods for these wastes (HSDB 2004). According to the TRI, 2,893 pounds of carbon tetrachloride were transferred to landfills and/or other treatment/disposal facilities and 617,050 pounds were sent to publicly owned treatment works in 2002 (TRI02 2004) (see Section 6.2).

Table 5-1. Facilities that Produce, Process, or Use Carbon Tetrachloride

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
AL	10	0	49,999,999	1, 3, 4, 5, 10, 11, 12, 13
AR	10	100	999,999	6, 7, 10, 11, 12
CA	25	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12
CO	8	1,000	999,999	1, 2, 5, 6, 10, 11, 12, 13
CT	2	10,000	999,999	6, 10
DE	2	100,000	999,999	6, 10
FL	1	10,000	99,999	12
GA	4	0	9,999,999	7, 8, 10, 11
ID	1	0	99	2, 3, 10, 11
IL	14	0	9,999,999	1, 3, 4, 5, 6, 7, 9, 10, 12
IN	13	0	9,999,999	1, 2, 3, 5, 10, 11, 12, 13, 14
KS	14	0	9,999,999	1, 3, 4, 6, 7, 10, 11, 12, 13
KY	12	1,000	49,999,999	1, 3, 5, 6, 9, 10, 12
LA	60	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13
MD	5	10,000	999,999	2, 3, 6, 7
MI	10	0	9,999,999	5, 6, 7, 10, 12
MN	4	100	99,999	7, 10, 11, 12
MO	5	100	99,999	7, 12
MS	6	0	999,999	8, 9, 10, 12
MT	2	100	99,999	10
NC	2	0	9,999	1, 7, 13
ND	2	1,000	99,999	10
NE	2	10,000	999,999	12
NH	1	0	99	12
NJ	16	0	9,999,999	1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12
NY	6	1,000	999,999	9, 10, 12, 13
OH	18	1,000	999,999	1, 4, 5, 9, 10, 11, 12, 13
OK	2	10,000	999,999	10
PA	10	1,000	9,999,999	6, 7, 10, 11, 12
PR	1	10,000	99,999	12
SC	7	1,000	9,999,999	6, 7, 8, 12
TN	14	0	999,999	1, 2, 3, 6, 7, 9, 10, 11, 12, 13, 14
TX	52	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
UT	1	10,000	99,999	12
VA	4	0	99,999	12
VI	3	10,000	9,999,999	10
WA	3	1,000	99,999	10, 11, 12
WI	2	1,000	9,999	9, 10

Table 5-1. Facilities that Produce, Process, or Use Carbon Tetrachloride

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
WV	11	0	9,999,999	1, 4, 5, 9, 11, 12, 13
WY	4	0	99,999	2, 3, 10

Source: TRI02 2004 (Data are from 2002)

[a]Post office state abbreviations used
[b]Amounts on site reported by facilities in each state
[c]Activities/Uses:

1. Produce
2. Import
3. Onsite use/processing
4. Sale/Distribution
5. Byproduct
6. Impurity
7. Reactant
8. Formulation Component
9. Article Component
10. Repackaging
11. Chemical Processing Aid
12. Manufacturing Aid
13. Ancillary/Other Uses
14. Process Impurity

6. POTENTIAL FOR HUMAN EXPOSURE

6.1 OVERVIEW

Carbon tetrachloride has been identified in at least 430 of the 1,662 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (HazDat 2005). However, the number of sites evaluated for carbon tetrachloride is not known. The frequency of these sites can be seen in Figure 6-1. Of these sites, 425 are located within the United States, 1 is located in Guam, 2 are located in the Virgin Islands, and 2 are located in the Commonwealth of Puerto Rico (not shown).

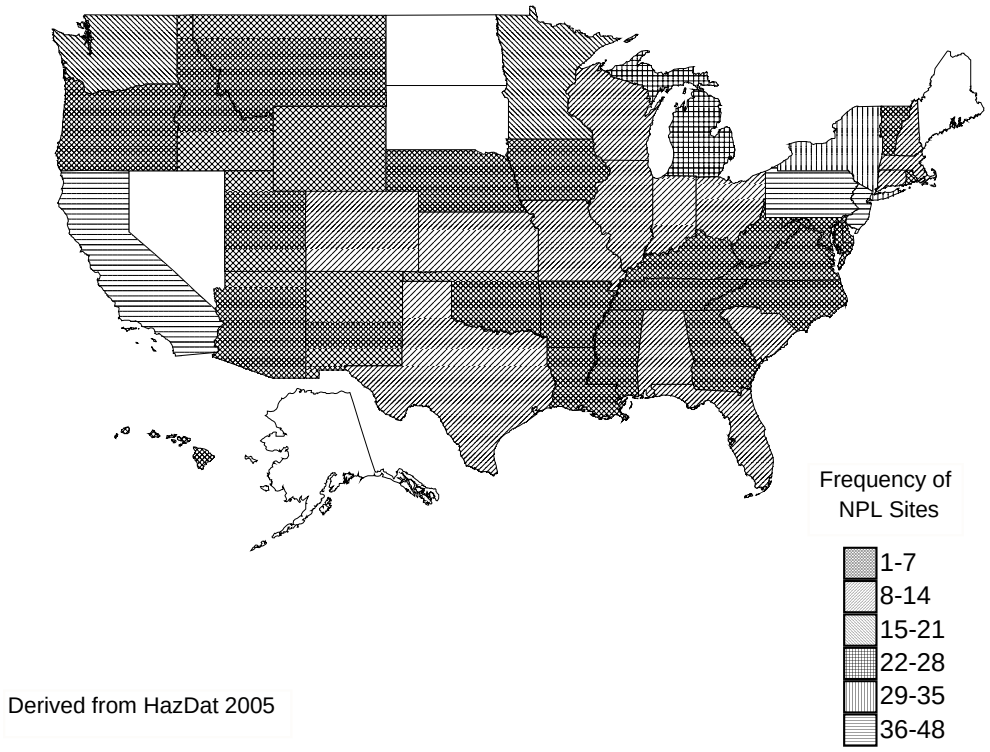
Carbon tetrachloride is a stable chemical that is degraded very slowly, so there has been a gradual accumulation of carbon tetrachloride in the environment as a consequence of releases from human activities. Until 1986, the largest source of release was from the use of carbon tetrachloride as a grain fumigant, but this practice has now been stopped. Other releases of carbon tetrachloride may occur during carbon tetrachloride production or during the use of carbon tetrachloride in the manufacture of chlorofluorocarbons and other chemical products.

Because carbon tetrachloride is volatile at ambient temperature, most carbon tetrachloride in the environment exists in the air. Typical levels in rural areas are about 1 $\mu g/m^3$, with somewhat higher values in urban areas and near industrial sources (Brodzinski and Singh 1983; Simmonds et al. 1983; Wallace et al. 1986). Low levels of carbon tetrachloride have been detected in many water systems (particularly surface water systems), with typical values of <0.5 µg/L (Letkiewicz et al. 1983). Less than 1% of all groundwater-derived drinking water systems has levels of carbon tetrachloride >0.5 µg/L and <0.2% have levels >5 mg/L (EPA 1987a).

6.2 RELEASES TO THE ENVIRONMENT

The TRI data should be used with caution because only certain types of facilities are required to report (EPA 1997). This is not an exhaustive list. Manufacturing and processing facilities are required to report information to the Toxics Release Inventory only if they employ 10 or more full-time employees; if their facility is classified under Standard Industrial Classification (SIC) codes 20–39; and if their facility

Figure 6-1. Frequency of NPL Sites with Carbon Tetrachloride Contamination



produces, imports, or processes ≥25,000 pounds of any TRI chemical or otherwise uses >10,000 pounds of a TRI chemical in a calendar year (EPA 1997).

6.2.1 Air

Estimated releases of 4.44 million pounds (202 metric tons) of carbon tetrachloride to the atmosphere from 55 domestic manufacturing and processing facilities in 2002, accounted for about 71% of the estimated total environmental releases from facilities required to report to the TRI (TRI02 2004). These releases are summarized in Table 6-1.

Although sources of carbon tetrachloride including marine algae, oceans, volcanoes, and drill wells have been cited (Gribble 1994), the majority of carbon tetrachloride in the environment is due to direct release to the atmosphere during production, disposal, or use of the compound. The estimated annual global release of carbon tetrachloride was about 60,000–80,000 metric tons/year during the period 1965–1977 (Singh et al. 1979a). Based on measurements of the rate of change of carbon tetrachloride levels in air around the globe, the calculated total atmospheric releases of carbon tetrachloride during the period 1978–1985 were around 90,000 metric tons/year (Simmonds et al. 1988). Some carbon tetrachloride may also be formed in air by photochemical decomposition of perchloroethylene (Singh et al. 1975) or by incomplete combustion of this chemical during waste incineration (Katami et al. 1992), although the magnitude of this contribution is difficult to estimate (Singh et al. 1979a).

Releases of carbon tetrachloride to air in the United States from manufacturing and processing ranged from 3.7 to 4.6 million pounds during 1987–1989, but were substantially reduced in 1990 and years after (EPA 1990, 1991b; TRI02 2004). According to the TRI02 (2004), an estimated total of 444,436 pounds (202 metric tons) of carbon tetrachloride, amounting to 71% of the total environmental release, was discharged to the air from manufacturing and processing facilities in the United States in 2001 (TRI02 2004) (see Table 6-1). The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list.

6.2.2 Water

Estimated releases of 320 pounds (0.145 metric tons) of carbon tetrachloride to surface water from 55 domestic manufacturing and processing facilities in 2002, accounted for about <1% of the estimated

Table 6-1. Releases to the Environment from Facilities that Produce, Process, or Use Carbon Tetrachloride[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
AL	1	10	0	0	0	0	0	10	10
AR	3	4,031	0	0	0	0	0	4,031	4,031
CA	1	1,000	0	0	0	0	0	1,000	1,000
IL	1	11	0	0	215	168	383	11	394
IN	2	500	250	0	5	0	0	755	755
KS	1	12,239	0	32,922	0	0	0	45,161	45,161
KY	2	854	0	0	5	0	5	854	859
LA	12	304,984	55	139,323	45	38	44	444,401	444,445
MD	1	119	0	0	0	0	0	119	119
MI	1	250	0	0	0	0	0	250	250
MS	1	500	0	0	0	0	0	500	500
NC	1	41	0	0	0	0	0	41	41
NE	1	255	0	0	0	0	0	255	255
OH	4	4,530	5	2	255	61	316	4,537	4,853
PA	2	6,983	0	0	500	1,564	2,064	6,983	9,047
TN	2	21	0	0	0	0	0	21	21
TX	15	107,605	10	5,568	8	28	5,604	107,618	113,223
UT	1	65	0	0	0	0	0	65	65
WV	2	438	0	0	0	0	0	438	438
WY	1	No data	No data	No data	No data	No data	No data	No data	No data
Total	55	444,436	320	177,815	1,033	1,860	8,417	617,050	625,467

Source: TRI02 2004 (Data are from 2002)

[a]The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list. Data are rounded to nearest whole number.
[b]Data in TRI are maximum amounts released by each facility.
[c]Post office state abbreviations are used.
[d]Number of reporting facilities.
[e]The sum of fugitive and point source releases are included in releases to air by a given facility.
[f]Surface water discharges, waste water treatment-(metals only), and publicly owned treatment works (POTWs) (metal and metal compounds).
[g]Class I wells, Class II-V wells, and underground injection.
[h]Resource Conservation and Recovery Act (RCRA) subtitle C landfills; other on-site landfills, land treatment, surface impoundments, other land disposal, other landfills.
[i]Storage only, solidification/stabilization (metals only), other off-site management, transfers to waste broker for disposal, unknown
[j]The sum of all releases of the chemical to air, land, water, and underground injection wells.
[k]Total amount of chemical transferred off-site, including to POTWs.

RF = reporting facilities; UI = underground injection

total environmental releases from facilities required to report to the TRI (TRI02 2004). These releases are summarized in Table 6-1.

Relatively small amounts of carbon tetrachloride are released to water. The total in 1978 was estimated to be 2.5 metric tons, due almost entirely to discharges from carbon tetrachloride production facilities (Rams et al. 1979). Analysis of data from EPA's Storage and Retrieval (STORET) database for the early 1980s indicate that carbon tetrachloride was detectable in 5.5% of 1,343 industrial effluent samples (Staples et al. 1985). The median concentration of all samples was <5 μg/L. Carbon tetrachloride was also detected in leachates from industrial landfills at concentrations ranging from <10 to 92 μg/L (Brown and Donnelly 1988).

In 1989, approximately 320 pounds (0.145 metric tons) of carbon tetrachloride was released in the United States to surface waters (EPA 1991b). An estimated total of 178,135 pounds (81 metric tons) of carbon tetrachloride, amounting to about 28% of the total environmental release, was discharged to the water and underground injection (potential groundwater release) from manufacturing and processing facilities in the United States in 2002 (TRI02 2004, see Table 6-1).

6.2.3 Soil

Estimated releases of 1,033 pounds (0.47 metric tons) of carbon tetrachloride to soils from 55 domestic manufacturing and processing facilities in 2002, accounted for about 0.0016% of the estimated total environmental releases from facilities required to report to the TRI (TRI02 2004). An additional 1.78 million pounds (81 metric tons), constituting about 28% of the total environmental emissions, were released via underground injection (TRI02 2004). These releases are summarized in Table 6-1.

Release of carbon tetrachloride to soil during carbon tetrachloride production was estimated to be 200,000 pounds (92 metric tons) in 1978 (Letkiewicz et al. 1983). Other sources of carbon tetrachloride discharged to soil include wastes associated with production and use of chlorofluorocarbons, metal cleaning compounds, adhesives, paints and other products. Total emissions to soil were estimated to be 2.6 million pounds (1,200 metric tons) in 1978 (Letkiewicz et al. 1983). In 1989, approximately 1,800 pounds (0.8 metric tons) of carbon tetrachloride were released in the United States to land (EPA 1991b). An estimated total of 1,033 pounds (0.47 metric tons) of carbon tetrachloride, amounting to <1%

of the total environmental release, was discharged to the soil from manufacturing and processing facilities in the United States in 2002 (TRI02 2004).

6.3 ENVIRONMENTAL FATE

6.3.1 Transport and Partitioning

Nearly all carbon tetrachloride released to the environment exists in the atmosphere (73% is released to the atmosphere directly). Most of the carbon tetrachloride released to soil and water evaporates within a few days (EPA 1991b). Because carbon tetrachloride does not degrade readily in the atmosphere, significant global transport is expected. Although carbon tetrachloride is moderately soluble in water (800 mg/L at 20 °C) (Verschueren 1983), only about 1% of the total carbon tetrachloride in the environment exists dissolved in surface waters and oceans (Galbally 1976). This is attributable to the relatively high rate of volatilization of low molecular weight chlorinated hydrocarbons from water (Dilling 1977; Dilling et al. 1975). Because of this, carbon tetrachloride also tends to volatilize from tap water used for showering, bathing, cooking, and other household uses inside a home (McKone 1987; Tancrede et al. 1992).

Most carbon tetrachloride released to soil is expected to volatilize rapidly due to its high vapor pressure (91.3 mmHg at 20 °C) (Howard 1990; IARC 1979). A fraction of the carbon tetrachloride remaining in the soil may adsorb to the soil organic matter, based on a calculated soil sorption coefficient of 110 (log K_{oc} of 2.04) (Kenaga 1980). Nevertheless, carbon tetrachloride is expected to be moderately mobile in most soils, depending on the organic carbon content, and leaching to groundwater is possible (Howard 1990). Marine sediments high in organic matter tended to have higher concentrations of carbon tetrachloride than did sediments with lower organic matter (McConnell et al. 1975). The composition of the soil organic matter and the water content of the soil may also affect sorption of carbon tetrachloride (Rutherford and Chiou 1992; Rutherford et al. 1992). Experimentally determined K_{oc} values for sorption of carbon tetrachloride on soils with organic carbon contents of 1.49 and 0.66% were 143.6 and 48.89 (log K_{oc} = 2.16 and 1.69), respectively (Walton et al. 1992). The retardation factor of carbon tetrachloride in breakthrough sampling in groundwater ranged from 1.4 to 1.7, indicating that soil adsorption is a relatively minor fate process (Mackay et al. 1983). Retardation factors for carbon tetrachloride measured in a flow-through system studying sorption of organics to aquifer materials with very low organic carbon (0.07–0.025%) ranged from 1.10 to 1.46 (Larsen et al. 1992), confirming this conclusion.

There is little tendency for carbon tetrachloride to bioconcentrate in aquatic or marine organisms. Reported log bioconcentration factors (BCFs) were 1.24 and 1.48 in trout and bluegill sunfish, respectively (HSDB 2004; Neely et al. 1974; Pearson and McConnell 1975). However, the log octanol/water partition coefficient (log K_{ow}) of 2.64 for carbon tetrachloride (EPA 1984) suggests that bioaccumulation is at least possible under conditions of constant exposure and may occur in occupational settings or in people living at or near hazardous waste sites. No data were located on the biomagnification of carbon tetrachloride. However, since most animals readily metabolize and excrete carbon tetrachloride following exposure (see Section 3.4.3), biomagnification is not expected.

6.3.2 Transformation and Degradation

6.3.2.1 Air

Carbon tetrachloride is very stable in the troposphere (Cox et al. 1976; Lillian et al. 1975; Singh et al. 1980). This is primarily because carbon tetrachloride does not react with hydroxyl radicals that initiate breakdown and transformation reactions of other volatile hydrocarbons. In addition, carbon tetrachloride does not photodissociate in the troposphere because, in the vapor state, it has no chromophores that absorb light in those visible or near ultraviolet regions of the electromagnetic spectrum, which prevail in the troposphere (Davis et al. 1975). The rate of oxidation of carbon tetrachloride is thought to be so slow that its estimated tropospheric half-life exceeds 330 years (Cox et al. 1976). Ultimately, carbon tetrachloride that is not removed from the troposphere by rainfall (Pearson and McConnell 1975) diffuses upward into the stratosphere where it may be photodegraded by shorter wavelength ultraviolet light (185–225 nm) more prevalent in this region of the atmosphere to form the trichloromethyl radical and chlorine atoms (Molina and Rowland 1974). The rate of photodissociation begins to become important at altitudes >20 km, and increases as altitude increases (Molina and Rowland 1974). Estimates of the atmospheric lifetime (the overall persistence of carbon tetrachloride in the troposphere and the stratosphere combined) are variable, but most values range from 30 to 100 years (EPA 1991b; Molina and Rowland 1974; Simmonds et al. 1983, 1988; Singh et al. 1979a), with 50 years generally being accepted as the most reasonable value.

Chlorine atoms and other chlorine species formed by photodecomposition of carbon tetrachloride in the stratosphere can catalyze reactions that destroy ozone. As the manufacture of carbon tetrachloride for use

in chlorofluorocarbons is phased out according to an international agreement (EPA 1987e), the impact of carbon tetrachloride on atmospheric ozone is likely to decrease.

6.3.2.2 Water

Carbon tetrachloride dissolved in water does not photodegrade or oxidize in any measurable amounts (Howard et al. 1991). The rate of hydrolysis in water is second order with respect to carbon tetrachloride, but is extremely slow, with a calculated half-life of 7,000 years at a concentration of 1 ppm (Mabey and Mill 1978). The reported aqueous hydrolysis rate calculated from gas phase measurements was $<2x10^{-6}M^{-1}s^{-1}$ (Haag and Yao 1992), 1–2 orders of magnitude less than other chlorinated alkanes. Others have suggested that hydrolysis may be the cause of decreasing carbon tetrachloride concentrations with depth in the ocean (Lovelock et al. 1973). However, this observation might also be explained by the biodegradation of carbon tetrachloride, which occurs much more rapidly than hydrolysis, particularly under anaerobic conditions. Biodegradation may occur within 16 days under anaerobic conditions (Tabak et al. 1981). Based upon acclimated aerobic screening test data, the aqueous aerobic half-life of carbon tetrachloride was estimated to be 6–12 months (Howard et al. 1991). Based upon unacclimated anaerobic screening test data and acclimated aerobic sediment/aquifer grab sample data, the aqueous anaerobic half-life of carbon tetrachloride was estimated to be 7–28 days (Howard et al. 1991).

The carbon atom in carbon tetrachloride is in its most oxidized state, therefore it is much more likely to undergo reductive degradation, as opposed to oxidative degradation (McCarty 1996a; McCarty and Reinhart 1993; McCarty and Semprini 1994; McCarty et al. 1996b). Carbon tetrachloride may undergo reductive dechlorination in aquatic systems in the presence of free sulfide and ferrous ions, or naturally occurring minerals providing those ions (Kriegman-King and Reinhard 1991). The transformation rate of carbon tetrachloride to chloroform and other products under simulated groundwater conditions at 50 °C was evaluated for the chemical alone, with minerals (biotite and vermiculite) providing ferrous ions and free sulfide ions, and with natural iron sulfides (pyrite and marcasite). Reported half-lives for carbon tetrachloride were 380 days for carbon tetrachloride alone, 2.9–4.5 days with minerals and sulfide ion present, and 0.44–0.85 days in the presence of natural iron sulfides. The effects noted with free ferrous or free sulfide ions were two orders of magnitude less than with natural minerals. Another recent study found degradation of 84% of the carbon tetrachloride present in aqueous solution containing ferrous ions 33 days, but no effect with sulfide ions (Doong and Wu 1992). Additional studies indicated that the abiotic reductive dechlorination of carbon tetrachloride could involve microbial cofactors or metabolites. Reductive dechlorination also occurs by anaerobic microbial transformation (Edwards et al. 1942).

Carbon tetrachloride removal via reductive dechlorination has also been observed under sulfate reducing conditions in an anaerobic system (de Best et al. 1998). Complete removal of carbon tetrachloride was observed, with chloroform and dichloromethane as the main transformation products; however, some unknown degradation products were also observed.

6.3.2.3 Sediment and Soil

No studies were located on the degradation of carbon tetrachloride in soil or sediment. Based on the estimated aqueous aerobic biodegradation half-life of carbon tetrachloride, the half-life of carbon tetrachloride in soil is estimated to be 6–12 months (Howard et al. 1991).

6.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

Reliable evaluation of the potential for human exposure to carbon tetrachloride depends in part on the reliability of supporting analytical data from environmental samples and biological specimens. Concentrations of carbon tetrachloride in unpolluted atmospheres and in pristine surface waters are often so low as to be near the limits of current analytical methods. In reviewing data on carbon tetrachloride levels monitored or estimated in the environment, it should also be noted that the amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable. The analytical methods available for monitoring carbon tetrachloride in a variety of environmental media are detailed in Chapter 7.

6.4.1 Air

Carbon tetrachloride appears to be ubiquitous in ambient air. Based on analysis of 4,913 ambient air samples reported in the National Ambient Volatile Organic Compounds Database (including remote, rural, suburban, urban, and source dominated sites in the United States), the average concentration of carbon tetrachloride was 0.168 ppb (1.1 μg/m^3) (Shah and Heyerdahl 1988). Carbon tetrachloride was detected in air at 76 NPL hazardous waste sites (HazDat 2005). Average values reported in four U.S. cities ranged from 0.144 to 0.291 ppb (Singh et al. 1992). Similar results were reported by Simmonds et al. (1983), who found average concentrations of 0.6–0.8 μg/m^3 (0.10–0.13 ppb) at five coastal monitoring stations around the world, and Kelly et al. (1994), who reported a median ambient

concentration of 0.8 μg/m^3 based on a compilation of ambient data from 1964 though 1992. Continued monitoring studies by Simmonds et al. (1988) reveal that global atmospheric levels of carbon tetrachloride have been steadily increasing by about 1.3% per year, reaching 0.12–0.14 ppb by 1985. Similar concentrations of carbon tetrachloride were also reported in air at five hazardous waste sites and one landfill in New Jersey, where average values ranged from 0.02 to 0.12 ppb (LaRegina et al. 1986). A study done involving the Toxic Air Monitoring System (TAMS) network showed concentrations of carbon tetrachloride in urban locations in Boston, Chicago, Houston, and the Seattle/Tacoma area (Evans et al. 1992). The median 24-hour concentrations were 0.12, 0.13, and 0.13 ppb at the three Boston sites, 0.12, 0.12, and 0.13 ppb at the three Chicago sites, 0.15, 0.13, and 0.12 ppb at the three Houston sites, and 0.12 ppb at the Seattle/Tacoma site. Sweet and Vermette (1990, 1992) have shown that carbon tetrachloride is present in areas of urban Illinois including southeast Chicago and east St. Louis at average concentrations of 0.7–1.0 μg/m^3 (0.11–0.16 ppb). It was determined in this study that point sources of carbon tetrachloride from industry and wind direction are responsible for localized increases in concentration. The Arizona hazardous air pollutants monitoring program has demonstrated average concentrations of carbon tetrachloride ranging from 0.7 to 0.75 μg/m^3 (0.11–0.12 ppb) (Zielinska et al. 1998). A study on air toxics in Minnesota has shown a carbon tetrachloride median concentration of 0.77 μg/m^3 (0.12 ppb) This concentration exceeded health benchmark values in 88% of monitoring sites (Pratt et al. 2000). A study monitoring levels of carbon tetrachloride in 13 urban areas of the United States during September 1996 to August 1997 provided a mean concentration of 0.072–0.09 ppbv throughout the areas (Mohamed et al. 2002). The more recent studies demonstrate a decrease in levels of carbon tetrachloride in the ambient air, which could be a reflection of the current drop in production; however, the resistance to atmospheric degradation allows for levels to remain somewhat constant. Ambient air concentrations of carbon tetrachloride were unaffected by changes in temperature and season (Mohamed et al. 2002), or day of the week (Austin 2003).

Studies have revealed that carbon tetrachloride is also a common contaminant of indoor air. Typical concentrations in homes in several U.S. cities were about 1 μg/m^3 (0.16 ppb), with some values up to 9 μg/m^3 (1.4 ppb) (Wallace et al. 1986). Concentrations in indoor air were usually higher than in outdoor air, indicating that the source of the carbon tetrachloride was building materials or products (pesticides, cleaning agents) inside the home (Wallace et al. 1986, 1987). Based on 2,120 indoor air samples in the United States, the average concentration of carbon tetrachloride was 0.4 ppb (2.6 μg/m^3) (Shah and Heyerdahl 1988). However, the median value was 0 ppb, indicating that carbon tetrachloride was not detected in more than half of the samples. A later study determined backyard outdoor air concentrations of carbon tetrachloride taken from 175 home sites in 6 urban areas to be 0.6 μg/m^3 (Wallace 1991). In

this same study, 24-hour average exposures of 750 people in 6 urban areas were determined to be 1 μg/m^3 (0.16 ppb). This indicates that for carbon tetrachloride, outdoor sources account for a majority of the airborne risk; however, indoor sources are still a concern (Acquavella et al. 1994; Wallace 1991). These data may reflect the effects of the discontinuation of the use of carbon tetrachloride in consumer products.

6.4.2 Water

There have been a number of surveys performed by the federal government to define typical levels of carbon tetrachloride in water supplies in this country. The results of these studies reveal that about 99% of all groundwater supplies and about 95% of all surface water supplies contain <0.5 μg/L of carbon tetrachloride (Letkiewicz et al. 1983). Carbon tetrachloride was detected in groundwater at 310 NPL hazardous waste sites, and in surface water at 53 NPL hazardous waste sites (HazDat 2005). Analysis of 945 drinking water samples from cities around the United States found detectable levels (>0.2 μg/L) in 30 (3.2%) of the samples (Westrick et al. 1984). The highest value reported was 16 μg/L, and the median value of the positive samples ranged from 0.3 to 0.7 μg/L in different sample groups. Carbon tetrachloride has also been detected in some private drinking water wells, at levels ranging from 1 to 720 μg/L (RIDOH 1989). Based on a survey of groundwater monitoring data from 479 waste sites, carbon tetrachloride was also detectable in groundwater (concentration not reported) at 32 sites in 9 EPA regions (Plumb 1991, 1992). A U.S. Geological Survey study of pesticide compounds present in well water around the United States showed the presence of carbon tetrachloride in <5% of the wells, but no concentration data were provided (Kolpin et al. 1997). A study on chemicals in California drinking water from 1984 to 1990 showed organic pollutants in 921 of 7,712 wells sampled (Lam et al. 1994). Of these contaminated wells, 45 were contaminated with carbon tetrachloride, at a maximum concentration of 29 μg/L (Lam et al. 1994). A survey of data by the National Academy of Sciences (NAS 1978) reported a range of carbon tetrachloride concentrations in seawaters of 0.2–0.7 ng/L. Based on analysis of data from the STORET database, carbon tetrachloride was detectable in 12% of 8,858 ambient water samples (Staples et al. 1985). The median concentration in all samples was 0.1 μg/L.

6.4.3 Sediment and Soil

Because carbon tetrachloride is ubiquitous in air, it is likely that trace levels of carbon tetrachloride are present in surface soils around the globe. Carbon tetrachloride was detected in soil at 103 NPL hazardous waste sites, and in sediment at 23 NPL hazardous waste sites (HazDat 2005). Based on information from

the STORET database, carbon tetrachloride was detected in 0.8% of sediment samples across the United States (Staples et al. 1985). The median concentration of all samples was <5 mg/kg dry weight.

6.4.4 Other Environmental Media

Until 1986, one of the major uses of carbon tetrachloride was as a fumigant for grain, and consequently, low levels of carbon tetrachloride occurred in grain or food products derived from such grain. Estimates of carbon tetrachloride residue levels in treated grain varied as a function of fumigation conditions and the amount of aeration after fumigation, but values of 1–100 mg/kg were typical (Deer et al. 1987; Letkiewicz et al. 1983; Lynn and Vorches 1957; McMahon 1971). Levels in finished food prepared from fumigated grains were considerably lower, with typical concentrations below 0.1 mg/kg (Berck 1974). Carbon tetrachloride was detected in 44 of 549 food items at an average concentration of 0.031 mg/kg in a Food and Drug Administration (FDA) survey (Daft 1991). However, carbon tetrachloride is no longer used for this purpose in the United States, so exposure from this source is no longer of concern, but certain foods may absorb small amounts of carbon tetrachloride from the air during processing (Daft 1991). Carbon tetrachloride does not appear to occur in significant quantities in most other foods (Letkiewicz et al. 1983; McConnell et al. 1975).

Carbon tetrachloride was detected in 11 of 1,159 household cleaning and related products in a survey conducted during the late 1980s (Sack et al. 1992). Since this chemical is no longer used in consumer products, exposure from this source is not likely to be of concern.

6.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

Members of the general population are most likely to be exposed to carbon tetrachloride through ambient air and drinking water. Despite being banned from consumer products, the long lifetime of carbon tetrachloride in the atmosphere contributes to the background level to which the general population is exposed (Wallace 1991). Assuming inhalation of 20 m^3/day by a 70-kg adult and 40% absorption of carbon tetrachloride across the lung (IRIS 2003), typical levels of carbon tetrachloride in ambient air (about 1 μg/m^3) yield systemically absorbed doses of about 0.1 μg/kg/day. Somewhat higher exposures could occur near point sources such as industries that produce or use carbon tetrachloride or hazardous waste sites contaminated with carbon tetrachloride. Estimates of daily intake from air and water range from 12 to 511 μg/day and from 0.2 to 60 μg/day, respectively, based on average concentrations of 0.1–

4 ppb (0.64–25.6 μg/m^3) in air and 0.1–30 μg/L in water (Howard 1990). For water, consumption of 2 L/day by a 70-kg adult containing a typical carbon tetrachloride concentration of 0.5 μg/L yields a typical daily intake of about 0.01 μg/kg/day.

A study by Hartwell et al. (1992) analyzed the levels of carbon tetrachloride breath, personal air, and fixed indoor and outdoor sites in the Los Angeles area of California. The percentages of samples in which carbon tetrachloride was detected overnight, during the winter season were 2.13% in breath, 81.4% in personal air, 90.5% in kitchen, and 91.3% in outdoor air. Based on these results, carbon tetrachloride is considered often found, but not at relatively high concentrations in the winter season, and therefore, concentrations were not provided. Similar results were determined for daytime and summer months.

Exposure to carbon tetrachloride may also occur by dermal and inhalation routes while using tap water for bathing and other household purposes (McKone 1987; Tancrede et al. 1992).

Exposure to carbon tetrachloride via food is not likely to be of significance, since levels in most foods are below analytical detection limits. Ingestion of bread or other products made with carbon tetrachloride-fumigated grain may have contributed to dietary exposure in the past, but this route of exposure is no longer believed to be of significance.

In the workplace, the most likely route of exposure is by inhalation. Air concentrations at a number of locations where fumigated grain was stored were well below 5 ppm, while some samples contained over 60 ppm (Deer et al. 1987). The average exposure of workers in the grain facilities ranged from 0.002 to 0.1 ppm, depending on job activity. For a worker exposed to 0.1 ppm (630 μg/m^3), the intake during an 8-hour day corresponds to a dose of about 35 μg/kg/day. Based on results of the National Occupational Exposure Survey (NOES) conducted during 1981–1983, the National Institute for Occupational Safety and Health (NIOSH) estimated that 58,208 workers were potentially exposed to carbon tetrachloride in the United States at that time (HSDB 2004). A study showing the baseline for potential emissions in the extrusion of polycarbonate resin at 304 °C showed that carbon tetrachloride was either undetectable or present at very low levels (Rhodes et al. 2002).

6.6 EXPOSURES OF CHILDREN

This section focuses on exposures from conception to maturity at 18 years in humans. Differences from adults in susceptibility to hazardous substances are discussed in Section 3.7, Children's Susceptibility.

Children are not small adults. A child's exposure may differ from an adult's exposure in many ways. Children drink more fluids, eat more food, breathe more air per kilogram of body weight, and have a larger skin surface in proportion to their body volume. A child's diet often differs from that of adults. The developing human's source of nutrition changes with age: from placental nourishment to breast milk or formula to the diet of older children who eat more of certain types of foods than adults. A child's behavior and lifestyle also influence exposure. Children crawl on the floor, put things in their mouths, sometimes eat inappropriate things (such as dirt or paint chips), and spend more time outdoors. Children also are closer to the ground, and they do not use the judgment of adults to avoid hazards (NRC 1993).

Young children often play close to the ground and frequently play in the dirt, which increases their dermal exposure to toxicants in dust and soil. They also tend to ingest soil, either intentionally through pica, or unintentionally through hand-to-mouth activity. Children, thus, may be orally dosed and dermally exposed to carbon tetrachloride present as a contaminant in soil and dust. It has been demonstrated that carbon tetrachloride vapors are absorbed by the skin slowly (HSDB 2004). In addition, carbon tetrachloride has a log K_{oc} value (organic carbon-water partition coefficient) of 2.04 (Kenaga 1980) indicating that it is not expected to adsorb to soil and sediment (HSDB 2004). Most of the carbon tetrachloride in the upper layers of the soil will be rapidly volatilized to air (vapor pressure=90 mmHg at 20 °C). Loss of carbon tetrachloride from the soil decreases the potential of dermal and oral exposure to children, but its rapid volatilization results in inhalation being the most likely route of exposure during play on the ground.

Children breathe in more air per kilogram of body weight than adults. Therefore, a child in the same micro-environment as an adult is likely to be exposed to a higher dosage of carbon tetrachloride from ambient air. Young children are closer to the ground or floor because of their height. The carbon tetrachloride vapors being heavier than air (vapor density=5.32, air=1, HSDB 2004) tend to concentrate near the ground. Children are therefore at a greater risk of exposure than adults during accidental spills or through indoor use of carbon tetrachloride in an unventilated area.

Exposures of the embryo or fetus to volatile organic compounds such as carbon tetrachloride may occur if the expectant mother is exposed. A newborn infant may be exposed by breathing contaminated air and by ingestion of mother's milk, which can contain small amounts of carbon tetrachloride. Children may be exposed through accidental ingestion of products containing carbon tetrachloride. Because of the toxicity of carbon tetrachloride, consumer uses have been discontinued, and only industrial uses remain (Section 5.3); therefore, the occurrence of products containing carbon tetrachloride being in the home should be low. Older children and adolescents may be exposed to carbon tetrachloride in their jobs or hobbies, or through deliberate solvent abuse by "sniffing." Inhalant abuse during pregnancy poses significant risks to the pregnancy and endangers both the mother and the fetus. Solvent abuse of carbon tetrachloride for euphoric effects would result in exposure levels that exceed those producing adverse effects in animals.

A study has been done in the Kanawha Valley in West Virginia observing children from 74 elementary schools in the this area (Ware et al. 1993). The Kanawha Valley region is one of the largest areas of chemical manufacturing in the United States. Concentrations of 5 petroleum-related compounds and 10 compounds more specific to industrially related processes, including carbon tetrachloride, were determined at the different schools in groups based on proximity to industry. It was determined that the mean concentration values of both the petroleum-related compounds and the process-related compounds for schools in the valley, near the chemical companies, were higher than for schools in the valley further away from the chemical companies, as well as schools out of the valley, both near and further away from the chemical companies. These values (19.71 $\mu g/m^3$ for the petroleum-related compounds and 5 $\mu g/m^3$ for the process-related compounds) are also higher than normally found in outdoor air around the country. A correlation was drawn between these higher concentrations of chemicals and an increased incidence of respiratory symptoms, including asthma, wheeze-related symptoms, and symptoms characteristic of reactive airway disease. It should be noted, however, that these data are for mixtures of volatile organic compounds and are not specific to carbon tetrachloride. Also, the observed data do not show direct causation of the observed symptoms; therefore, a need exists for further investigation of the effects of carbon tetrachloride on children (Donelly et al. 1995).

6.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Workers involved in the manufacture or use of carbon tetrachloride are the population most likely to have exposures to carbon tetrachloride significantly higher than members of the general public. Workers exposed to concentrations in air ranging from 20 to 125 ppm for intermediate durations have experienced

a variety of neurological effects (see Section 3.2.1.4). Current regulations restrict the acceptable concentration of carbon tetrachloride in workplace air to 2 ppm, but this is still much higher than commonly encountered in the ambient environment. Fugitive emissions of carbon tetrachloride from chemical plants may expose area residents to elevated levels of this halocarbon, although concentrations outside the plant are typically much lower than in the chemical plant itself. Other populations that might have above average exposure include persons living near hazardous waste sites contaminated with carbon tetrachloride.

6.8 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of carbon tetrachloride is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of carbon tetrachloride.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.8.1 Identification of Data Needs

Physical and Chemical Properties. The physical and chemical properties of carbon tetrachloride have been well studied, and reliable values for key parameters are available for use in environmental fate and transport models. On this basis, it does not appear that further studies of the physical-chemical properties of carbon tetrachloride are essential.

Production, Import/Export, Use, Release, and Disposal. According to the Emergency Planning and Community Right-to-Know Act of 1986, 42 U.S.C. Section 11023, industries are required to submit substance release and off-site transfer information to the EPA. The TRI, which contains this information

for 2002, became available in May of 2004. This database is updated yearly and should provide a list of industrial production facilities and emissions.

Although the production of carbon tetrachloride has been declining, humans are at risk of exposure to the compound at specific industrial locations where the compound is used or near chemical waste sites where emission to the environment may occur. Available data indicate that most carbon tetrachloride manufactured in this country is consumed in the synthesis of chlorofluorocarbons, but current quantitative data on the amounts of carbon tetrachloride imported and exported into and from the United States are sparse (HSDB 2004; USITC 2003). According to the Emergency Planning and Community Right-to Know Act of 1986, 43 U.S.C. Section 11023, Industries are required to submit substance release and off-site transfer information to the EPA. TRI, which contains this information for 2002, became available in 2004. This database is updated yearly and should provide a list of industrial production facilities and emissions.

In 2002, the United States released approximately 414,000 pounds (188 metric tons) of carbon tetrachloride to the environment from manufacturing and processing facilities, most of which (70%) was released directly to the atmosphere (TRI02 2004). Carbon tetrachloride is considered a hazardous waste and is subject to disposal regulations. Information on current disposal practices for used containers, sludges, and soils containing carbon tetrachloride waste are lacking. Because carbon tetrachloride is so stable in the environment, collection of this information on production, use, release, and disposal are needed to evaluate the effect of current industrial practices on local and global levels of carbon tetrachloride. Further, this information would be useful in the overall evaluation of human health risk of carbon tetrachloride.

Environmental Fate. The environmental fate of carbon tetrachloride has been investigated by a number of workers, and available data are adequate to conclude that one main fate process is volatilization followed by photodecomposition in the stratosphere (Pearson and McConnell 1975). However, there is some uncertainty in available estimates of atmospheric lifetime, and more detailed studies of the rate of carbon tetrachloride decomposition, and how this depends on altitude, geographic location, and other atmospheric components, are needed to refine models predicting global and local trends in carbon tetrachloride levels. Although only a small fraction of environmental carbon tetrachloride is thought to exist in surface waters, the possibility exists that hydrolysis, bioaccumulation, or adsorption, while slow, could compete with the slow photodecomposition occurring in the atmosphere. Estimates on the aerobic and anaerobic biodegradation half-lives of carbon tetrachloride in water have

been made based on limited data. For this reason, additional studies on carbon tetrachloride flux rates into and out of surface water, as well as refined quantitative estimates of aquatic fate processes would be valuable. The chemical is expected to evaporate rapidly from soil due to its high vapor pressure and may migrate into groundwater due to its low soil adsorption coefficient. No data are available on biodegradation in soil. Additional studies to determine degradation rates and the extent to which adsorption has occurred are needed. These data are also useful in evaluating the impact of carbon tetrachloride leaching from hazardous waste sites.

Bioavailability from Environmental Media. Carbon tetrachloride can be absorbed following oral dosing and inhalation, or dermal exposure. No data were located regarding the potential effects of environmental media (air, water, soil) on the absorption of carbon tetrachloride. However, since soil adsorption is considered to be relatively low for carbon tetrachloride, it seems unlikely that soil would have a significant effect on its bioavailability. Additional studies are needed to determine the extent of bioavailability from contaminated air, drinking water, and soil at hazardous waste sites.

Food Chain Bioaccumulation. Limited data indicate that carbon tetrachloride has a low tendency to bioconcentrate in the food chain, even though it is a lipophilic compound (Neely et al. 1974; Pearson and McConnell 1975). The lack of bioconcentration is mainly due to the volatility of carbon tetrachloride, which facilitates clearance from exposed organisms. Nevertheless, carbon tetrachloride does tend to become concentrated in fatty tissues, and further studies on the levels of carbon tetrachloride in the fat of fish would help evaluate the risk of carbon tetrachloride exposure by this pathway. No data are available on the bioconcentration in plants. Additional studies would be useful in assessing potential for human exposure from ingestion of plant foodstuff. Data are also needed on the biomagnification of the compound in the aquatic and terrestrial food chain. These data would be useful in assessing food chain bioaccumulation as a potential human exposure pathway.

Exposure Levels in Environmental Media. Reliable monitoring data for the levels of carbon tetrachloride in contaminated media at hazardous waste sites are needed so that the information obtained on levels of carbon tetrachloride in the environment can be used in combination with the known body burden of carbon tetrachloride to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

Levels of carbon tetrachloride in air, water, and sediments have been measured at numerous locations in the United States, and typical or average exposure levels in ambient air and drinking water are fairly well

defined (Letkiewicz et al. 1983; Shah and Heyerdahl 1988; Singh et al. 1992; Westrick et al. 1984). There is considerable local variation, with higher-than-average levels occurring in some industrial areas and near some waste sites. However, much of this information is no longer current. Consequently, further monitoring of carbon tetrachloride in the workplace and in ambient water and air near known or potential sources of carbon tetrachloride would be valuable in identifying locations where human exposure could be elevated.

Reliable monitoring data for the levels of carbon tetrachloride in contaminated media at hazardous waste sites are needed so that the information obtained on levels of carbon tetrachloride in the environment can be used in combination with the known body burden of carbon tetrachloride to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

Exposure Levels in Humans. Detection of carbon tetrachloride in blood, urine, and expired air has been used as an indicator of exposure to the compound in occupational settings. Similar information on the general population, particularly in the vicinity of hazardous waste sites, are needed to estimate levels of the compound to which the general population has been exposed and perhaps some correlation of these levels with levels of carbon tetrachloride in contaminated air, drinking water, and soil.

This information is necessary for assessing the need to conduct health studies on these populations.

Exposures of Children. There are very limited data on the effects of carbon tetrachloride exposure on children. As stated earlier (Section 6.6), adult data cannot simply be extrapolated to children for a variety of different reasons. Data on children's exposure are needed.

Child health data needs relating to susceptibility are discussed in Section 3.12.2, Identification of Data Needs: Children's Susceptibility.

Exposure Registries. No exposure registries for carbon tetrachloride were located. This substance is not currently one of the compounds for which a sub-registry has been established in the National Exposure Registry. The substance will be considered in the future when chemical selection is made for sub-registries to be established. The information that is amassed in the National Exposure Registry facilitates the epidemiological research needed to assess adverse health outcomes that may be related to exposure to this substance.

6.8.2 Ongoing Studies

As part of the Third National Health and Nutrition Evaluation Survey (NHANES III), the Environmental Health Laboratory Sciences Division of the National Center for Environmental Health, Centers for Disease Control and Prevention, will be analyzing human blood samples for carbon tetrachloride and other volatile organic compounds. These data will give an indication of the frequency of occurrence and background levels of these compounds in the general population.

The Federal Research in Progress (FEDRIP 2004) database provides additional information obtainable from a few ongoing studies that may fill in some of the data needs identified in Section 6.8.2. These studies that include one which deals with the phytoremediation of toxic waste sites using poplar trees to remove toxic solvents from influent waters. Over a 4-year period, 98–99% of trichloroethylene was removed, with similar results shown for carbon tetrachloride and perchloroethylene. Further study is being done using this methodology to determine the fate of the carbon from these chemicals.

7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring carbon tetrachloride, its metabolites, and other biomarkers of exposure and effect to carbon tetrachloride. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision.

As is true for most volatile organic compounds, the preferred analytical technique for carbon tetrachloride is gas chromatography (GC). A number of devices are suitable for detection of carbon tetrachloride as it emerges from the GC, including flame ionization detector (FID), halogen-sensitive detector (HSD), or electron-capture detector (ECD). In general, HSD or ECD are preferable because of their high sensitivity for halogenated compounds. When absolute confidence in compound identity is required, gas chromatography/mass spectrometry (GC/MS) is the method of choice.

The most variable aspect of carbon tetrachloride analysis is the procedure used to extract carbon tetrachloride from the medium and prepare a sample suitable for GC analysis. As a volatile organic compound of relatively low water solubility, carbon tetrachloride is easily lost from biological and environmental samples, so appropriate care must be exercised in handling and storing such samples for chemical analysis. Brief summaries of the methods available for extraction and detection of carbon tetrachloride in biological and environmental samples are provided below.

7.1 BIOLOGICAL MATERIALS

Separation of carbon tetrachloride from biological samples may be achieved by headspace analysis, purge-and-trap collection from aqueous solution or slurry samples, solvent extraction, or direct collection on resins. Headspace analysis offers speed, simplicity, and good reproducibility, but partitioning of the

analyte between the headspace and the sample matrix is dependent upon the nature of the matrix and must be determined separately for each different kind of matrix (Walters 1986).

Purge-and-trap collection is well adapted to biological samples such as blood or urine that are soluble in water (Pellizzari et al. 1985a; Peoples et al. 1979), and is readily adapted from techniques that have been developed for the analysis of carbon tetrachloride in water and waste water. For water-insoluble materials, the purge-trap approach is complicated by uncertainty of partitioning the analyte between sample slurry particles and water.

Historically, diethyl ether has been a widely used solvent for the extraction of volatile components from biological fluids (Zlatkis and Kim 1976). Homogenization of tissue with the extractant and lysing of cells improves extraction efficiency. When, as is often the case, multiple analytes are being determined using solvent extraction, selective extraction and loss of low-boiling compounds can cause errors. Highly purified solvents have largely eliminated problems with solvent impurities, although high costs, solvent toxicities, and restrictions on spent solvent disposal must be considered. Supercritical fluid extraction using pure carbon dioxide or carbon dioxide with additives offers some potential for the extraction of organic analytes such as carbon tetrachloride from biological samples (Hawthorne 1988).

Analytical methods for the determination of carbon tetrachloride in biological samples are summarized in Table 7-1.

7.2 ENVIRONMENTAL SAMPLES

The basic method for collection of carbon tetrachloride from the ambient atmosphere is adsorption on a solid phase, followed by removal by thermal or solvent elution for subsequent analysis. One of the most common adsorbents for carbon tetrachloride is Tenax® GC. Using Tenax® adsorbent, standard air containing 1.15 ppb by gas volume of carbon tetrachloride was determined with biases of -23.0, -34.7, -50.0, and -69.2% at collection volumes of 10, 20, 38, and 76 L of air, respectively (Crist and Mitchell 1986). Citing these large negative biases even when the sampled volume was less than 10% of the breakthrough volume, these authors conclude that Tenax® is not suitable for quantitative sampling for carbon tetrachloride (Crist and Mitchell 1986). For occupational monitoring of carbon tetrachloride in air, NIOSH (1984) recommends samplers containing activated carbon. The adsorbed carbon tetrachloride is extracted from the activated carbon with carbon disulfide, then determined by GC/FID. Studies have been conducted to improve analytical methods for detection of low-level volatile organic compounds.

Table 7-1. Analytical Methods for Determining Carbon Tetrachloride in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Alveolar air	Collect on Tenax-TA®; desorb thermally; inject by cryotrap	Capillary column GC/FID	No data	No data	Clair et al. 1991
Breath	Collect on Tenax-GC®; desorb thermally	Capillary column GC/MS	No data	No data	Pellizzari et al. 1985b
Adipose tissue	Purge from liquefied fat at 115 °C, trap on Tenax ®/silica gel, thermal desorption	GC/HSD	<1.3 μg/L	96 (90–100)	Peoples et al. 1979
Adipose tissue	Macerate in water; purge with inert gas; trap on Tenax-GC®; desorb thermally	Capillary column GC/MS	≈6 ng/g	≈50	Pellizzari et al. 1985a
Blood serum	Purge from water-serum mixture containing antifoam reagent at 115 °C, trap on Tenax®/silica gel, thermal desorption	GC/HSD	<1.3 μg/L	112 (108–124)	Peoples et al. 1979
Blood	Purge with inert gas, trap on Tenax-GC®; desorb thermally	Capillary column GC/MS	≈3 ng/mL	89.4	Pellizzari et al. 1985a
Biofluids	Dilute with water, sealed vial; collect headspace vapors	GC/FID	NR	No data	Suitheimer et al. 1982

FID = flame ionization detector; GC = gas chromatography; HSD = halogen-selective detector; MS = mass spectrometry; NR = not reported

Methods have been evaluated that do not require the use of sorbents, thereby reducing associated uncertainties due to their adsorption/desorption efficiencies. The use of cryogenic preconcentration techniques to increase the sample content of trace volatile toxic organic compounds in a gas matrix for analysis by GC has been evaluated (Rhoderick and Miller 1990). The authors revealed that a linear multipoint calibration range from 1 to 15 ppb can be obtained by using a single standard, cryogenic trapping, a constant flow rate and varied trapping timer. Acceptable methods for the determination of carbon tetrachloride in ambient air are detailed in EPA Compendium Method TO-14A (EPA 1999).

Purge and trap methods are standard for the determination of carbon tetrachloride in water, with analyte measurement by GC using halogen-specific detection, electron-capture detection, or mass-spectrometry (APHA 1992a, 1992b; ASTM 1987; Bellar 1989; Eichelberger and Buddle 1989a, 1989b; EPA 1982a, 1982b; Ho 1989). The APHA (1992a, 1992b) methods for carbon tetrachloride have been accepted by EPA as equivalent to EPA-developed methods. Analyte measurement using an ion trap detector that functions as a mass spectrometer has also been evaluated (Eichelberger et al. 1990). This method is sufficiently sensitive to measure the analytes below the regulatory levels. Headspace sampling, coupled with whole column cryotrapping chromatography and mass spectrometry, have been used in the analysis of volatile priority pollutants in water and waste water (Gryder-Boutlet and Kennish 1988). The advantage of headspace sampling over other methods of analysis include minimal sample preparation, injection of a larger sample preparation and, and shorter analysis timer, because all of the compounds being analyzed are volatile. Carbon tetrachloride can also be determined in solid wastes by purge and trap collection followed by GC (EPA 1986a, 1986b). A modified open-loop dynamic headspace technique has been applied for stripping and trapping volatile organic compounds from estuarine sediments (Bianchi et al. 1991). This method is capable of quantifying volatile organic compounds at detection limits between 10 and 100 ng/kg.

Analytical methods for the determination of carbon tetrachloride in environmental samples are summarized in Table 7-2.

7.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of carbon tetrachloride is available. Where adequate

Table 7-2. Analytical Methods for Determining Carbon Tetrachloride in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Coconut shell carbon sorption, carbon disulfide desorption	GC/FID	10 µg/sample	No data	NIOSH 1984
Air	Adsorption on Tenax® GC, thermal desorption	GC/MS	<1.15 ppb[a]	23–77	Crist and Mitchell 1986
Air	Sorption	GC/CLMD	0.003 ng/ sample	No data	Yamada et al. 1982
Air	Charcoal sorption, carbon disulfide desorption	GC/HSD	No data	No data	ASTM 1987
Water	Purge and trap	GC/MS	No data	No data	ASTM 1987
Water	Extract with n-pentane	GC/ECD	0.4 µg/L	No data	Garcia et al. 1992
Water	Purge and trap	GC/HSD	0.12 µg/L	82.5	EPA 1982a
Water	Purge and trap	GC/MS	2.8 µg/L	102	EPA 1982b
Drinking water	Purge with inert gas; trap on sorbent tube; desorb thermally	Capillary column GC/HSD	0.01 µg/L	92	Ho 1989
Drinking water	Purge with inert gas; trap on sorbent tube; desorb thermally	Packed column GC/HSD	0.003 µg/L	90	Bellar 1989
Drinking water	Purge with inert gas; trap on sorbent tube; desorb thermally	Capillary column GC/HSD	0.08 µg/L	92	EPA 1989b
Drinking water	Purge with inert gas; trap on sorbent tube; desorb thermally	Packed column GC/HSD	0.3 µg/L	88	EPA 1989b
Water	Purge and trap	GC/ITD	0.1 µg/L	No data	Eichelberger et al. 1990
Water	Solvent extraction (isooctane)	GC/ECD	1 µg/L[b]	No data	ASTM 1988
Soil	Purge and trap	GC/HSD	1.2 µg/kg	43–143	EPA 1986b
Wastes, non-water miscible	Purge and trap	GC/HSD	150 µg/kg	43–143	EPA 1986b
Solid waste	Purge and trap	GC/MS	5 µg/kg	70–140	EPA 1986a
Grain	Extract with acetone/water (5/1); dry; inject acetone solution	GC/ECD	NR	No data	AOAC 1984

[a]Persistent negative bias in recovery suggests Tenax® sorption is not suitable for collection of carbon tetrachloride.
[b]Approximate detection limit

CLMD = chemiluminescence detection; ECD = electron capture detector; FID = flame ionization detector; GC = gas chromatography; HSD = halogen-selective detector; ITD = ion trap detector; MS = mass spectrometry; NR = not reported

information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of carbon tetrachloride.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. Covalent adducts between reactive carbon tetrachloride metabolites (e.g., the trichloromethyl radical) and cellular proteins, lipids and nucleic acids are known to occur, but at present these can only be measured using radiolabeled carbon tetrachloride. Development of immunological or other methods to detect such adducts in humans exposed to carbon tetrachloride could be of value in estimating past exposures to carbon tetrachloride.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Analytical methods are available for measuring carbon tetrachloride in air, water, soil, and solid waste, and most of these methods have good sensitivity and specificity (APHA 1992a, 1992b; ASTM 1987; Bellar 1989; Eichelberger and Buddle 1989a, 1989b; EPA 1982a, 1982b; Ho 1989). However, the estimated 10-6 cancer risk levels for carbon tetrachloride are quite low (0.01 ppb in air and 0.3 ppb in drinking water) (IRIS 2003), so improvements in sensitivity would be valuable. It is desirable to have means to measure organohalides such as carbon tetrachloride *in situ* in water and other environmental media. One approach to doing this has been demonstrated by the *in situ* analysis of chloroform-contaminated well water using remote fiber fluorimetry (RFF) and fiber optic chemical sensors (FOCS) (Milanovich 1986). With this approach, fluorescence of basic pyridine in the presence of an organohalide (Fujiwara reaction) is measured from a chemical sensor immersed in the water at the end of an optical fiber. Carbon tetrachloride undergoes a Fujiwara reaction, so its determination might be amenable to this approach. Another *in situ* method for field monitoring of carbon tetrachloride has been described by Kirtland et al. (2003); this method uses isotopic labeling and detection of metabolites by gas chromatography.

7.3.2 Ongoing Studies

The EPA is funding on-going research to develop a "Master Analytical Scheme" for organic compounds in water (Michael et al. 1988), which includes carbon tetrachloride as an analyte. The overall goal is to detect organic compounds at 0.1 µg/L in drinking water, 1 µg/L in surface water, and 10 µg/L in effluent waters. Analytes are to include numerous semivolatile compounds and some compounds that are only "semisoluble" in water, as well as volatile compounds. A comprehensive review of the literature leading up to these efforts has been published (Pellizzari et al. 1985a).

Improvements in analytical technology to identify groundwater contaminants revealed that soil gas analysis may enhance the effectiveness of traditional sampling and analysis (Kerfoot 1990). Carbon tetrachloride has properties that make it amenable to detection by soil gas analysis.

Researchers have coupled two GC capillary columns with different lengths and polarities in series to optimize separation of complex mixtures of volatile organics in air samples (Clair et al. 1991). Atomic emission detectors (AEDs) and mass selective detectors (MSDs) are also being used to enhance selectivity and sensitivity for air analyses (Yamashita et al. 1992).

The Environmental Health Laboratory Sciences Division of the Center for Environmental Health and Injury Control, Centers for Disease Control, is developing methods for the analysis of carbon tetrachloride and other volatile organic compounds in blood (Ashley et al. 1992). These methods use purge and trap methodology, high resolution gas chromatography, and magnetic mass spectrometry which gives detection limits in the low parts per trillion (ppt) range. Also useful is the ability to test for carbon tetrachloride and other volatile organic compounds in expired air (Wallace 1996).

8. REGULATIONS AND ADVISORIES

Because of its potential to cause adverse health effects in exposed people, a number of regulations and advisory values have been established for carbon tetrachloride by various international, national, and state agencies. These values are summarized in Table 8-1.

ATSDR has calculated an intermediate inhalation MRL of 0.03 ppm based on a NOAEL of 5 ppm and a LOAEL of 10 ppm for liver effects in an intermediate-duration (187–192 days) inhalation study in rats exposed 7 hours/day, 5 days/week (Adams et al. 1952). The intermediate-duration MRL is expected to be protective also for acute-duration inhalation exposures. ATSDR has also calculated a chronic inhalation MRL of 0.03 ppm based on a NOAEL of 5 ppm and a LOAEL of 25 ppm for hepatic effects (increased liver weight, serum enzymes, and liver histopathology) in rats exposed for 6 hours/day, 5 days/week for 2 years (Japan Bioassay Research Center 1998; Nagano et al. 1998). ATSDR has also calculated an acute oral MRL of 0.02 mg/kg/day based on a LOAEL of 5 mg/kg/day over 10 days for minimal liver effects (vacuolar degeneration) in the rat (Smialowicz et al. 1991), and an intermediate oral MRL of 0.007 mg/kg/day based on a NOAEL of 1 mg/kg/day (0.71 mg/kg/day adjusted for intermittent exposure) and a LOAEL of 10 mg/kg/day for liver effects in rats dosed 5 days/week over 12 weeks (Bruckner et al. 1986). More information about the derivation of MRLs is found in Section 2.3 and Appendix A.

EPA has calculated a chronic oral reference dose (RfD) of $7x10^{-4}$ mg/kg/day for carbon tetrachloride based on a NOAEL of 1 mg/kg/day (converted to 0.71 mg/kg/day based on intermittent exposure) for rats in a 12-week study (Bruckner et al. 1986; IRIS 2003). The critical effect was liver toxicity. A subchronic oral RfD of $7x10^{-3}$ mg/kg/day was also calculated based on the same NOAEL used for the chronic RfD (EPA 1989b). It should be noted that EPA is currently developing new assessments for carbon tetrachloride that have not yet been released for public review.

Table 8-1. Regulations and Guidelines Applicable to Carbon Tetrachloride

Agency	Description	Information	Reference
INTERNATIONAL			
Guidelines:			
IARC	Carcinogenicity classification	Group 2B[a]	IARC 1999
WHO	Guideline value or tolerable concentration for air quality	6.1 µg/m^3	WHO 2000
	Guideline for drinking water	2 µg/L	WHO 1993
NATIONAL			
Regulations and Guidelines:			
a. Air			
ACGIH	TLV (8-hour TWA)[b]	5 ppm	ACGIH 2003
	TLV-STEL (15-minute TWA)	10 ppm	
EPA	Hazardous air pollutant pursuant to Section 112 of the Clean Air Act	Yes	EPA 2003e 40 CFR 61.01
	Protection of stratospheric ozone; listed as a ozone-depleting chemical	Group IV	EPA 2003h 40 CFR 82, Subpart A, Appendix F
NIOSH	STEL (60-minute TWA)	2 ppm	NIOSH 2003
	IDLH	200 ppm	
	Potential occupational carcinogen	Yes	
OSHA	PEL (8-hour TWA) for general industry	2 mg/m^3	OSHA 2003c 29 CFR 1910.1000, Table Z-1
	PEL (8-hour TWA) Acceptable ceiling concentration Acceptable maximum peak above the acceptable ceiling concentration for an 8-hour shift	10 ppm 25 ppm 200 ppm (maximum duration for 5 minutes in any 4 hours)	OSHA 2003e 29 CFR 1910.1000, Table Z-2
	PEL (8-hour TWA) for construction industry[c]	10 ppm	OSHA 2003f 29 CFR 1926.55, Appendix A
	PEL (8-hour TWA) for shipyard industry[c]	10 ppm	OSHA 2003a 29 CFR 1915.1000
USC	Hazardous air pollutant	Yes	USC 2003 42 USC 7412
b. Water			
EPA	Drinking water health advisories		EPA 2002
	1-day (10-kg child)	4 mg/L	
	10-day (10-kg child)	0.2 mg/L	
	DWEL[d]	0.03 mg/L	
	10^{-4} Cancer risk[e]	0.03 mg/L	
	Effluent guidelines and standards; toxic pollutants pursuant to Section 307(a)(1) of the Clean Water Act	Yes	EPA 2003c 40 CFR 401.15
	Hazardous substance in accordance with Section 311 of the Clean Water Act	Yes	EPA 2003n 40 CFR 116.4

Table 8-1. Regulations and Guidelines Applicable to Carbon Tetrachloride

Agency	Description	Information	Reference
	National primary drinking water regulations—MCL	5 µg/L	EPA 2003g 40 CFR 141.61
NATIONAL *(cont.)*			
EPA	National primary drinking water regulations—MCLG	0 µg/L	EPA 2003f 40 CFR 141.50
	Pollutant of initial focus in the Great Lakes Water Quality Initiative	Yes	EPA 2003o 40 CFR 132, Table 6
	Reportable quantity of hazardous substances designated pursuant to Section 311 of the Clean Water Act	10 pounds	EPA 2003i 40 CFR 117.3
c. Food			
FDA	Bottled drinking water allowable level	5 µg/L	FDA 2003a 21 CFR 165.110
	Indirect food additive; adhesives	Yes	FDA 2003b 21 CFR 175.105(c)(5)
	Indirect food additive; paper and paperboard components; anti-offset substances	Yes	FDA 2003c 21 CFR 176.130(c)
	Indirect food additive; components of paper and paperboard in contact with dry food	Yes	FDA 2003d 21 CFR 176.180(b)(2)
	Labeling; warning statements for prescription and restricted device products containing or manufactured with chlorofluorocarbons or other ozone-depleting substances	Yes	FDA 2003f 21 CFR 801.433
	Labeling; medical devices; warning statements for devices containing or manufactured with chlorofluorocarbons and other class I ozone-depleting substances	Yes	FDA 2003e 21 CFR 801.63
d. Other			
ACGIH	Carcinogenicity classification	A2[f]	ACGIH 2003
EPA	Carcinogenicity classification	B2[g]	IRIS 2003
	RfC	No data	IRIS 2003
	RfD (chronic oral)	$7x10^{-4}$ mg/kg/day	IRIS 2003
	Community right-to-know; release reporting; effective date of reporting	01/01/87	EPA 2003m 40 CFR 372.65
	Criteria for municipal solid waste landfills; hazardous constituent	Yes	EPA 2003a 40 CFR 258, Appendix II
	Identification and listing of hazardous waste; regulatory level of the maximum concentration of contaminants for the toxicity characteristic	0.5 mg/L	EPA 2003d 40 CFR 261.24

Table 8-1. Regulations and Guidelines Applicable to Carbon Tetrachloride

Agency	Description	Information	Reference
NATIONAL *(cont.)*			
EPA	Reportable quantity; designated as a hazardous substances pursuant to Section 307 and 311 of the Clean Water Act, Section 112 of the Clean Air Act, and Section 3001 of RCRA	10 pounds	EPA 2003b 40 CFR 302.4
	Standards for owners and operators of hazardous waste TSD facilities; groundwater monitoring	Suggested Method PQL 8010 1 µg/L 8240 5 µg/L	EPA 2003l 40 CFR 264, Appendix IX
	Standards for owners and operators of hazardous waste TSD facilities; health-based limits for exclusion of waste-derived residues; residue concentration limit	$5x10^{-3}$ mg/kg	EPA 2003k 40 CFR 266, Appendix VII
	Standards for the management of specific hazardous waste and hazardous waste management facilities; risk specific dose	$6.7x10^{-1}$ µg/m^3	EPA 2003j 40 CFR 266, Appendix V
NTP	Carcinogenicity classification	Reasonably anticipated to be a human carcinogen	NTP 2002
STATE			
a. Air	No data		
b. Water			
Arizona	Drinking water guideline	0.27 µg/L	
California	Drinking water standard	0.5 µg/L	HSDB 2003
Connecticut	Drinking water guideline	5 µg/L	HSDB 2003
Florida	Drinking water standard	3 µg/L	HSDB 2003
Maine	Drinking water guideline	2.7 µg/L	HSDB 2003
Minnesota	Drinking water guideline	3 µg/L	HSDB 2003
New Jersey	Drinking water standard	2 µg/L	HSDB 2003
c. Food	No data		

Table 8-1. Regulations and Guidelines Applicable to Carbon Tetrachloride

Agency	Description	Information	Reference
STATE *(cont.)*			
d. Other	No data		

[a]Group 2B: possibly carcinogenic to humans
[b]Skin notation: refers to the potential significant contribution to the overall exposure by the cutaneous route, including mucous membranes and the eyes, either by contact with vapors or, of probable greater significance, by direct skin contact with the substance.
[c]Skin designation
[d]DWEL: a lifetime exposure concentration protection of adverse, non-cancer health effects, that assumes all of the exposure to a contaminant is from drinking water.
[e]10^{-2} Cancer risk: the concentration of a chemical in drinking water corresponding to an excess estimated lifetime cancer risk of 1 in 10,000.
[f]A2: suspected human carcinogen
[g]B2: probable human carcinogen

ACGIH = American Conference of Governmental Industrial Hygienists; CFR = Code of Federal Regulations; DWEL = drinking water equivalent level; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; HSDB = Hazardous Substances Data Bank; IARC = International Agency for Research on Cancer; IDLH = immediately dangerous to life or health; IRIS = Integrated Risk Information System; MCL = maximum contaminant level; MCLG = maximum contaminant level goal; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; PQL = practical quantitation limit; OSHA = Occupational Safety and Health Administration; PEL = permissible exposure limit; RCRA = Resource Conservation and Recovery Act; RfC = inhalation reference concentration; RfD = oral reference dose; STEL = short-term exposure limit; TLV = threshold limit values; TSD = treatment, storage, and disposal; TWA = time-weighted average; USC = United States Codes; WHO = World Health Organization

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10. GLOSSARY

Absorption—The taking up of liquids by solids, or of gases by solids or liquids.

Acute Exposure—Exposure to a chemical for a duration of 14 days or less, as specified in the Toxicological Profiles.

Adsorption—The adhesion in an extremely thin layer of molecules (as of gases, solutes, or liquids) to the surfaces of solid bodies or liquids with which they are in contact.

Adsorption Coefficient (K_{oc})—The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

Adsorption Ratio (Kd)—The amount of a chemical adsorbed by sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

Benchmark Dose (BMD_{10})—Usually defined as the lower confidence limit on the dose that produces a specified magnitude of changes in a specified adverse response. For example, a BMD10 would be the dose at the 95% lower confidence limit on a 10% response, and the benchmark response (BMR) would be 10%. The BMD is determined by modeling the dose response curve in the region of the dose response relationship where biologically observable data are feasible.

Benchmark Dose Model—A statistical dose-response model applied to either experimental toxicological or epidemiological data to calculate a BMD.

Bioconcentration Factor (BCF)—The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

Biomarkers—Broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility.

Cancer Effect Level (CEL)—The lowest dose of chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or tumors) between the exposed population and its appropriate control.

Carcinogen—A chemical capable of inducing cancer.

Case-Control Study—A type of epidemiological study that examines the relationship between a particular outcome (disease or condition) and a variety of potential causative agents (such as toxic chemicals). In a case-controlled study, a group of people with a specified and well-defined outcome is identified and compared to a similar group of people without outcome.

Case Report—Describes a single individual with a particular disease or exposure. These may suggest some potential topics for scientific research, but are not actual research studies.

Case Series—Describes the experience of a small number of individuals with the same disease or exposure. These may suggest potential topics for scientific research, but are not actual research studies.

Ceiling Value—A concentration of a substance that should not be exceeded, even instantaneously.

Chronic Exposure—Exposure to a chemical for 365 days or more, as specified in the Toxicological Profiles.

Cohort Study—A type of epidemiological study of a specific group or groups of people who have had a common insult (e.g., exposure to an agent suspected of causing disease or a common disease) and are followed forward from exposure to outcome. At least one exposed group is compared to one unexposed group.

Cross-sectional Study—A type of epidemiological study of a group or groups of people that examines the relationship between exposure and outcome to a chemical or to chemicals at one point in time.

Data Needs—Substance-specific informational needs that if met would reduce the uncertainties of human health assessment.

Developmental Toxicity—The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Dose-Response Relationship—The quantitative relationship between the amount of exposure to a toxicant and the incidence of the adverse effects.

Embryotoxicity and Fetotoxicity—Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the insult occurs. The terms, as used here, include malformations and variations, altered growth, and *in utero* death.

Environmental Protection Agency (EPA) Health Advisory—An estimate of acceptable drinking water levels for a chemical substance based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

Epidemiology—Refers to the investigation of factors that determine the frequency and distribution of disease or other health-related conditions within a defined human population during a specified period.

Genotoxicity—A specific adverse effect on the genome of living cells that, upon the duplication of affected cells, can be expressed as a mutagenic, clastogenic, or carcinogenic event because of specific alteration of the molecular structure of the genome.

Half-life—A measure of rate for the time required to eliminate one half of a quantity of a chemical from the body or environmental media.

Immediately Dangerous to Life or Health (IDLH)—The maximum environmental concentration of a contaminant from which one could escape within 30 minutes without any escape-impairing symptoms or irreversible health effects.

Immunologic Toxicity—The occurrence of adverse effects on the immune system that may result from exposure to environmental agents such as chemicals.

Immunological Effects—Functional changes in the immune response.

Incidence—The ratio of individuals in a population who develop a specified condition to the total number of individuals in that population who could have developed that condition in a specified time period.

Intermediate Exposure—Exposure to a chemical for a duration of 15–364 days, as specified in the Toxicological Profiles.

***In Vitro*—**Isolated from the living organism and artificially maintained, as in a test tube.

***In Vivo*—**Occurring within the living organism.

Lethal Concentration(Lo) (LC_{Lo})—The lowest concentration of a chemical in air that has been reported to have caused death in humans or animals.

Lethal Concentration(50) (LC_{50})—A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose(Lo) (LD_{Lo})—The lowest dose of a chemical introduced by a route other than inhalation that has been reported to have caused death in humans or animals.

Lethal Dose(50) (LD_{50})—The dose of a chemical that has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time(50) (LT_{50})—A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL)—The lowest exposure level of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Lymphoreticular Effects—Represent morphological effects involving lymphatic tissues such as the lymph nodes, spleen, and thymus.

Malformations—Permanent structural changes that may adversely affect survival, development, or function.

Minimal Risk Level (MRL)—An estimate of daily human exposure to a hazardous substance that is likely to be without an appreciable risk of adverse noncancer health effects over a specified route and duration of exposure.

Modifying Factor (MF)—A value (greater than zero) that is applied to the derivation of a Minimal Risk Level (MRL) to reflect additional concerns about the database that are not covered by the uncertainty factors. The default value for a MF is 1.

Morbidity—State of being diseased; morbidity rate is the incidence or prevalence of disease in a specific population.

Mortality—Death; mortality rate is a measure of the number of deaths in a population during a specified interval of time.

Mutagen—A substance that causes mutations. A mutation is a change in the DNA sequence of a cell's DNA. Mutations can lead to birth defects, miscarriages, or cancer.

Necropsy—The gross examination of the organs and tissues of a dead body to determine the cause of death or pathological conditions.

Neurotoxicity—The occurrence of adverse effects on the nervous system following exposure to a chemical.

No-Observed-Adverse-Effect Level (NOAEL)—The dose of a chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Effects may be produced at this dose, but they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{ow})—The equilibrium ratio of the concentrations of a chemical in *n*-octanol and water, in dilute solution.

Odds Ratio (OR)—A means of measuring the association between an exposure (such as toxic substances and a disease or condition) that represents the best estimate of relative risk (risk as a ratio of the incidence among subjects exposed to a particular risk factor divided by the incidence among subjects who were not exposed to the risk factor). An OR of greater than 1 is considered to indicate greater risk of disease in the exposed group compared to the unexposed group.

Organophosphate or Organophosphorus Compound—A phosphorus-containing organic compound and especially a pesticide that acts by inhibiting cholinesterase.

Permissible Exposure Limit (PEL)—An Occupational Safety and Health Administration (OSHA) allowable exposure level in workplace air averaged over an 8-hour shift of a 40-hour workweek.

Pesticide—General classification of chemicals specifically developed and produced for use in the control of agricultural and public health pests.

Pharmacokinetics—The dynamic behavior of a material in the body, used to predict the fate (disposition) of an exogenous substance in an organism. Utilizing computational techniques, it provides the means of studying the absorption, distribution, metabolism, and excretion of chemicals by the body.

Pharmacokinetic Model—A set of equations that can be used to describe the time course of a parent chemical or metabolite in an animal system. There are two types of pharmacokinetic models: data-based and physiologically-based. A data-based model divides the animal system into a series of compartments, which, in general, do not represent real, identifiable anatomic regions of the body, whereas the physiologically-based model compartments represent real anatomic regions of the body.

Physiologically Based Pharmacodynamic (PBPD) Model—A type of physiologically based dose-response model that quantitatively describes the relationship between target tissue dose and toxic end points. These models advance the importance of physiologically based models in that they clearly describe the biological effect (response) produced by the system following exposure to an exogenous substance.

Physiologically Based Pharmacokinetic (PBPK) Model—Comprised of a series of compartments representing organs or tissue groups with realistic weights and blood flows. These models require a variety of physiological information: tissue volumes, blood flow rates to tissues, cardiac output, alveolar ventilation rates, and possibly membrane permeabilities. The models also utilize biochemical information, such as air/blood partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

Prevalence—The number of cases of a disease or condition in a population at one point in time.

Prospective Study—A type of cohort study in which the pertinent observations are made on events occurring after the start of the study. A group is followed over time.

q_1*—The upper-bound estimate of the low-dose slope of the dose-response curve as determined by the multistage procedure. The q_1* can be used to calculate an estimate of carcinogenic potency, the incremental excess cancer risk per unit of exposure (usually μg/L for water, mg/kg/day for food, and μg/m^3 for air).

Recommended Exposure Limit (REL)—A National Institute for Occupational Safety and Health (NIOSH) time-weighted average (TWA) concentration for up to a 10-hour workday during a 40-hour workweek.

Reference Concentration (RfC)—An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer health effects during a lifetime. The inhalation reference concentration is for continuous inhalation exposures and is appropriately expressed in units of mg/m^3 or ppm.

Reference Dose (RfD)—An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure of the human population to a potential hazard that is likely to be without risk of deleterious effects during a lifetime. The RfD is operationally derived from the no-observed-adverse-effect level (NOAEL, from animal and human studies) by a consistent application of uncertainty factors that reflect various types of data used to estimate RfDs and an additional modifying factor, which is based on a professional judgment of the entire database on the chemical. The RfDs are not applicable to nonthreshold effects such as cancer.

Reportable Quantity (RQ)—The quantity of a hazardous substance that is considered reportable under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Reportable quantities are (1) 1 pound or greater or (2) for selected substances, an amount established by regulation either under CERCLA or under Section 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

Reproductive Toxicity—The occurrence of adverse effects on the reproductive system that may result from exposure to a chemical. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

Retrospective Study—A type of cohort study based on a group of persons known to have been exposed at some time in the past. Data are collected from routinely recorded events, up to the time the study is undertaken. Retrospective studies are limited to causal factors that can be ascertained from existing records and/or examining survivors of the cohort.

Risk—The possibility or chance that some adverse effect will result from a given exposure to a chemical.

Risk Factor—An aspect of personal behavior or lifestyle, an environmental exposure, or an inborn or inherited characteristic that is associated with an increased occurrence of disease or other health-related event or condition.

Risk Ratio—The ratio of the risk among persons with specific risk factors compared to the risk among persons without risk factors. A risk ratio greater than 1 indicates greater risk of disease in the exposed group compared to the unexposed group.

Short-Term Exposure Limit (STEL)—The American Conference of Governmental Industrial Hygienists (ACGIH) maximum concentration to which workers can be exposed for up to 15 minutes continually. No more than four excursions are allowed per day, and there must be at least 60 minutes between exposure periods. The daily Threshold Limit Value-Time Weighted Average (TLV-TWA) may not be exceeded.

Standardized Mortality Ratio (SMR)—A ratio of the observed number of deaths and the expected number of deaths in a specific standard population.

Target Organ Toxicity—This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen—A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV)—An American Conference of Governmental Industrial Hygienists (ACGIH) concentration of a substance to which most workers can be exposed without adverse effect. The TLV may be expressed as a Time Weighted Average (TWA), as a Short-Term Exposure Limit (STEL), or as a ceiling limit (CL).

Time-Weighted Average (TWA)—An allowable exposure concentration averaged over a normal 8-hour workday or 40-hour workweek.

Toxic Dose($_{50}$) (TD$_{50}$)—A calculated dose of a chemical, introduced by a route other than inhalation, which is expected to cause a specific toxic effect in 50% of a defined experimental animal population.

Toxicokinetic—The absorption, distribution, and elimination of toxic compounds in the living organism.

Uncertainty Factor (UF)—A factor used in operationally deriving the Minimal Risk Level (MRL) or Reference Dose (RfD) or Reference Concentration (RfC) from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using lowest-observed-adverse-effect level (LOAEL) data rather than no-observed-adverse-effect level (NOAEL) data. A default for each individual UF is 10; if complete certainty in data exists, a value of 1 can be used; however, a reduced UF of 3 may be used on a case-by-case basis, 3 being the approximate logarithmic average of 10 and 1.

Xenobiotic—Any chemical that is foreign to the biological system.

APPENDIX A. ATSDR MINIMAL RISK LEVELS AND WORKSHEETS

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) [42 U.S.C. 9601 et seq.], as amended by the Superfund Amendments and Reauthorization Act (SARA) [Pub. L. 99–499], requires that the Agency for Toxic Substances and Disease Registry (ATSDR) develop jointly with the U.S. Environmental Protection Agency (EPA), in order of priority, a list of hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL); prepare toxicological profiles for each substance included on the priority list of hazardous substances; and assure the initiation of a research program to fill identified data needs associated with the substances.

The toxicological profiles include an examination, summary, and interpretation of available toxicological information and epidemiologic evaluations of a hazardous substance. During the development of toxicological profiles, Minimal Risk Levels (MRLs) are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. MRLs are based on noncancer health effects only and are not based on a consideration of cancer effects. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the no-observed-adverse-effect level/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (365 days and longer) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive chemical-induced end point considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals.

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology, expert panel peer reviews, and agency-wide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published levels. For additional information regarding MRLs, please contact the Division of Toxicology, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop F-32, Atlanta, Georgia 30333.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Carbon Tetrachloride
CAS Number: 56-23-5
Date: June 2005
Profile Status: Final Post-Public Comment Draft
Route: [X] Inhalation [ ] Oral
Duration: [ ] Acute [X] Intermediate [ ] Chronic
Graph Key: 30
Species: Rat

Minimal Risk Level: 0.03 [ ] mg/kg/day [X] ppm

Reference: Adams EM, Spencer HC, Rowe VK, et al. 1952. Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals. Arch Ind Hyg Occup Med 6:50-66.

Experimental design: Groups of Wistar rats (15–25 males, 15–23 females) were exposed to vapors of carbon tetrachloride (5, 10, 25, 50, 100, 200, and 400 ppm) for 173–205 days (5 days/week, 7 hours/day). Two kinds of control groups were used: 'unexposed controls' that were maintained in the animal quarters and 'air-exposed controls' that were exposed to room air while in exposure chambers at equivalent frequency and duration as compound-exposed animals. Animals were weighed twice weekly and observed frequently for clinical signs. Food consumption was monitored. Hematological (prothrombin time) and biochemical indices (blood urea nitrogen, phospholipid, esterified cholesterol) were monitored in selected groups. Gross necropsy was performed and organ weights were determined for lung, heart liver, kidneys, spleen, and testes. Histopathological examination was performed on these and 11 other organs.

Effects noted in study and corresponding doses: No effects were observed in the 5 ppm exposure groups for any of the parameters measured. In rats, fatty degeneration of the liver and increased liver weight were evident at concentrations of ≥10 ppm and hepatic cirrhosis and pathology of the renal tubular epithelium (moderate cloudy swelling) occurred at ≥50 ppm. At ≥200 ppm, hepatic necrosis, increased kidney weight, degeneration of the renal tubular epithelium, and some testicular atrophy were observed. The severity of effects increased with exposure level.

Dose and end point used for MRL derivation: The MRL was based on a NOAEL of 5 ppm and a LOAEL of 10 ppm for fatty degeneration and increased liver weights.

[X] NOAEL [ ] LOAEL

Uncertainty Factors used in MRL derivation:

[ ] 10 for use of a LOAEL
[X] 3 for extrapolation from animals to humans using a dosimetric adjustment
[X] 10 for human variability

Was a conversion used from ppm in food or water to a mg/body weight dose? Not applicable.
If so, explain:

If an inhalation study in animals, list the conversion factors used in determining human equivalent dose: A human equivalent concentration was calculated from the rat NOAEL of 5 ppm in the principal study for an extrarespiratory effect of a type 3 gas, as recommended by EPA (1994) guidance for derivation of inhalation reference concentrations. A human equivalent concentration of the identified rat NOAEL of 5 ppm ($NOAEL_{HEC}$) was calculated by multiplying the duration-adjusted rat NOAEL ($NOAEL_{ADJ}$) by the ratio of the rat and human blood:gas partition coefficients. The $NOAEL_{ADJ}$ is 0.9 ppm (5 ppm x 7 hours/24 hours x 5 days/7 days) and the blood:gas partition coefficient ratio is 1.7 (4.52/2.64). Because the ratio was greater than 1, a default value of 1 was applied, resulting in a $NOAEL_{HEC}$ of 0.9 ppm. An uncertainty factor of 30 was applied to the $NOAEL_{HEC}$ of 0.9 ppm (3 for extrapolation from animals to humans using a dosimetric adjustment and 10 for human variability).

Other additional studies or pertinent information which lend support to this MRL: Limited human data are available for intermediate-duration inhalation exposure to carbon tetrachloride. Effects in humans exposed intermittently included gastrointestinal effects (nausea, dyspepsia) at 20–50 ppm, central nervous system depression at 40 ppm, and narcosis at 80 ppm (Elkins 1942; Heiman and Ford 1941; Kazantzis and Bomford 1960). An occupational study of hepatic effects in workers exposed from <1 to >5 years indicated that serum levels of hepatic enzymes were significantly elevated only at exposures >1 ppm, but the actual durations of exposure were not reported (Tomenson et al. 1995). Interpretation of this study is also limited by the finding that the group estimated to have had the highest exposure did not show the highest levels of serum enzymes. The liver appears to be the most sensitive target in animals exposed for intermediate durations. Fatty degeneration, sometimes with increased liver weight, was observed at a LOAEL of 10 ppm in rats, mice, and guinea pigs treated 6–8 hours/day, 5 days/week for 12–36 weeks or continuously for 90 days (Adams et al. 1952; DOE 1999; Japan Bioassay Research Center 1998; Prendergast et al. 1967), and 50–100 ppm in monkeys (Adams et al. 1952; Smyth et al. 1936). Increased serum enzymes and necrosis were observed in mice at 20 ppm and hamsters at 100 ppm (DOE 1999). Exposure to higher concentrations resulted in cirrhosis in guinea pigs (25 ppm) and rats (50–270 ppm) (Adams et al. 1952; Japan Bioassay Research Center 1998; Prendergast et al. 1967; Smyth et al. 1936). In studies examining other organs, renal effects (tubular degeneration) were noted at 50–200 ppm in rats (Adams et al. 1952; Smyth et al. 1936), at 90 ppm in rats and mice (Japan Bioassay Research Center 1998), and at 200 ppm in monkeys (Smyth et al. 1936). Injury to sciatic and optical nerves was noted in rats at 50 ppm (Smyth et al. 1936); hematological effects (decreased erythrocytes, hemoglobin, hematocrit; hemolysis, increased spleen weight) were observed in rats and mice exposed to 90–270 ppm (Japan Bioassay Research Center 1998; Smyth et al. 1936), and reproductive toxicity (decreased litters, testicular atrophy) was noted at 200 ppm (Adams et al. 1952; Smyth et al. 1936). Hepatotoxicity is identified as the critical effect of intermediate-duration inhalation exposure to carbon tetrachloride since it was noted at the lowest LOAELs. The study by Adams et al. (1952) is selected as the principal study because it identified the lowest LOAEL and the highest NOAEL for the critical effect.

Agency Contacts (Chemical Managers): Obaid Faroon, Ph.D.; Jessilynn Taylor, M.S.; Nickolette Roney, M.P.H.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Carbon Tetrachloride
CAS Number: 56-23-5
Date: June 2005
Profile Status: Final Post-Public Comment Draft
Route: [X] Inhalation [ ] Oral
Duration: [ ] Acute [ ] Intermediate [X] Chronic
Graph Key: 50
Species: Rat

Minimal Risk Level: 0.03 [ ] mg/kg/day [X] ppm

References: Japan Bioassay Research Center. 1998. Subchronic inhalation toxicity and carcinogenicity studies of carbon tetrachloride in F344 rats and BDF1 mice (Studies Nos. 0020, 0021, 0043, and 0044). Kanagawa, Japan Industrial Safety and Health Association, Japan Bioassay Research Center (Unpublished report to the Ministry of Labor). Hirasawa Hadano Kanagawa, 257 Japan. (In 2001, T. Matsushima provided to SRC organ weight data tables for these studies.)

(Methods published in: Nagano K, Nishizawa T, Yamamoto S, et al. 1998. Inhalation carcinogenesis studies of six halogenated hydrocarbons in rats and mice. In: Chiyotani K, Hosoda Y, Aizawa Y, eds. Advances in the prevention of occupational respiratory diseases. Elsevier Science B.V., 741-746.)

Experimental design: Groups of 50 male and 50 female F344/DuCrj rats were exposed (whole-body) to vapors of carbon tetrachloride (>99% pure) at concentrations of 0, 5, 25, or 125 ppm, 6 hours/day, 5 days/week for 104 weeks. Rats were observed daily for clinical signs, behavioral changes, and mortality. Body weights were measured weekly for the first 13 weeks and every 4 weeks thereafter. Urinalysis was performed at the end of the dosing period. Hematology and serum chemistry were measured in blood samples taken during final euthanization after overnight fasting. All organs and tissues were examined for gross lesions, weighed, and fixed for histopathological analysis.

Effects noted in study and corresponding concentrations: Male rats at ≥5 ppm exhibited enhanced hemosiderin deposition in the spleen; this was apparently a residual effect of anemia that was observed in the 13-week study, but not at 104 weeks. No significant hepatic effects were noted at 5 ppm. At ≥25 ppm, significant hepatic effects were observed: statistically significant elevations relative liver weights, serum parameters (total bilirubin, ALT, AST), and increased incidences of liver histopathology (fatty change, granulation, foci in the liver, deposition of ceroid, and serious effects such as fibrosis and cirrhosis). Chronic nephropathy was observed in all groups, including controls, but at greater severity at 25 ppm and above; significant proteinuria (dipstick values of 3+ or 4+) was also observed in all groups (in >90% of controls), but at higher severity in males treated at 5 ppm and females at 25 ppm and above. At 25 ppm, females had significant hematological changes (decreased hemoglobin, hematocrit, and lymphocyte counts and increased leukocyte and segmented neutrophil counts). At 125 ppm, body weights were decreased and there was increased mortality from chronic nephrosis and tumors.

The tumors observed at 125 ppm included: hepatocellular adenomas in 21/50 males and 40/50 females and hepatocellular carcinomas in 32/50 males and 15/50 females. At 25 ppm, females had significant hematological changes (decreased hemoglobin, hematocrit, and lymphocyte counts and increased leukocyte and segmented neutrophil counts).

Dose and end point used for MRL derivation: A NOAEL of 5 ppm LOAEL of 25 ppm for increased liver weight, serum enzymes, and liver histopathology (fatty change, granulation, foci, deposition of ceroid, fibrosis, and cirrhosis).

[X] NOAEL [ ] LOAEL

Uncertainty Factors used in MRL derivation:

[ ] 10 for use of a LOAEL
[X] 3 for extrapolation from animals to humans using dosimetric adjustment
[X] 10 for human variability

Was a conversion used from ppm in food or water to a mg/body weight dose? Not applicable.
If so, explain:

If an inhalation study in animals, list the conversion factors used in determining human equivalent concentration: A human equivalent concentration was calculated from the rat NOAEL of 5 ppm in the principal study for an extrarespiratory effect of a type 3 gas, as recommended by EPA (1994) guidance for derivation of inhalation reference concentrations. A human equivalent concentration of the identified rat NOAEL of 5 ppm for hepatic effects (Japan Bioassay Research Center 1998) was calculated by multiplying the duration-adjusted rat NOAEL ($NOAEL_{ADJ}$) by the ratio of the rat and human blood:gas partition coefficients. The $NOAEL_{ADJ}$ is 0.9 ppm (5 ppm x 6 hours/24 hours x 5 days/7 days) and the blood:gas partition coefficient ratio is 1.7 (4.52/2.64). Because the ratio was greater than 1, a default value of 1 was applied, resulting in a $NOAEL_{HEC}$ of 0.9 ppm. An uncertainty factor of 30 was applied to the $NOAEL_{HEC}$ to derive the chronic-duration inhalation MRL.

Other additional studies or pertinent information which lend support to this MRL: The chronic-duration inhalation database for carbon tetrachloride includes the occupational study by Tomenson et al. (1995) and 2-year bioassays in rats and mice (Japan Bioassay Research Center 1998; Nagano et al. 1998). As discussed under the intermediate-duration MRL, elevated hepatic serum enzymes were observed in workers who had been exposed to concentrations >1 ppm for <1–>5 years, but the actual durations of exposure were not reported (Tomenson et al. 1995). Interpretation of this study is also limited by the finding that the group estimated to have had the highest exposure did not show the highest levels of serum enzymes. In the 2-year bioassay in BDF_1 mice, groups of 50/sex were treated at 0, 5, 25, or 125 ppm, 6 hours/day, 5 days/week for 104 weeks (Japan Bioassay Research Center 1998; Nagano et al. 1998). No effects were noted at the lowest concentration of 5 ppm. In mice, 25 ppm was a LOAEL for most observed effects: hematological (increased extramedullary hematopoeisis in spleen associated with recovery from anemia), body weight (reduced body weight gain), renal (protein casts and altered clinical chemistry values), and hepatic (increased liver weights, degeneration, cyst, deposition of ceroid, increased serum enzymes, cholesterol, bilirubin in both sexes, and thrombus and necrosis in females). Mice at ≥25 ppm also exhibited significant increases in the incidences of hepatic adenoma and carcinoma with increased mortality.

One effect in rat was noted at 5 ppm, but was not selected as the critical effect of chronic-duration inhalation exposure. The severity of proteinuria, but not renal histopathology, was elevated in male and female rats treated at 5 ppm compared to controls; however, as the severity in control rats was so high (>90% with scores of 3+ or 4+), this lesion was not used as the basis for MRL derivation. Hepatotoxicity is selected as the critical effect of chronic-duration inhalation exposure because the severity of effects at 25 ppm was greater compared to other end points. Furthermore, selection of hepatotoxicity as the critical effect of chronic exposure is consistent with the database for intermediate-duration inhalation exposure. The 2-year bioassay in rats is selected as the principal study for the chronic-duration inhalation MRL

since it provided a NOAEL of 5 ppm and a LOAEL of 25 ppm for hepatic effects without increased mortality.

Agency Contacts (Chemical Managers): Obaid Faroon, Ph.D.; Jessilynn Taylor, M.S.; Nickolette Roney, M.P.H.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Carbon Tetrachloride
CAS Number: 56-23-5
Date: June 2005
Profile Status: Final Post-Public Comment Draft
Route: [ ] Inhalation [X] Oral
Duration: [X] Acute [ ] Intermediate [ ] Chronic
Graph Key: 23
Species: Rat

Minimal Risk Level: 0.02 [X] mg/kg/day [ ] ppm

Reference: Smialowicz RJ, Simmons JE, Luebke RW, et al. 1991. Immunotoxicologic assessment of subacute exposure of rats to carbon tetrachloride with comparison to hepatotoxicity and nephrotoxicity. Fundam Appl Toxicol 17:186-196.

Experimental design: Groups of 5–6 male Fischer 344 rats were dosed by gavage for 10 consecutive days with 0, 5, 10, 20, or 40 mg/kg/day of carbon tetrachloride in corn oil. Serum chemistry profiles, hepatic cytochrome P-450 content and activity, and kidney and liver organ weight and histopathology were assessed. Various immune function parameters were also examined in these animals, and in another set exposed to 40, 80, or 160 mg/kg/day. Immune function end points included relative spleen and thymus weights; natural killer cell activity; lymphoproliferative response to concanavalin A, phytohemagglutinin, pokeweed mitogen, and *Salmonella typhimurium* mitogen; allogeneic cytotoxic T lymphocyte reaction; and primary antibody response to sheep red blood cells.

Effects noted in study and corresponding doses: No hepatic effects were observed in controls. Minimal centrilobular vacuolar degeneration was detectable in all rats at 5 mg/kg/day; degeneration was mild in all rats treated at 10 and 20 mg/kg/day and 5/6 rats at 40 mg/kg/day and moderate in one high-dose rat. Hepatocellular necrosis was minimal in 3/6 rats at 10 mg/kg/day, 5/6 rats at 20 mg/kg/day, and 5/6 rats at 40 mg/kg/day, and mild in one high-dose rat. Serum ALT and AST levels were significantly elevated 1.5–5.4-fold compared to controls at doses of 20 and 40 mg/kg/day. Mean relative liver weight was significantly ($p<0.01$) increased by 17.7% compared to controls at 40 mg/kg/day. Treatment with carbon tetrachloride had no significant effect compared to controls on body weight, absolute liver weight, or renal parameters at doses from 5 to 40 mg/kg/day. However, when three separate 40 mg/kg/day groups and their controls were analyzed by two-way ANOVA with carbon tetrachloride and replicates as factors, a significant decrease in weight gain was detected. Body weight gain was significantly reduced at 80 mg/kg/day and higher, as determined by comparison of the slopes of weight gains over the dosing period. There were no adverse effects on immunological parameters at doses up to 160 mg/kg/day.

Dose and end point used for MRL derivation: LOAEL of 5 mg/kg/day for minimal vacuolar degeneration of centrilobular hepatocytes.

[ ] NOAEL [X] LOAEL

Uncertainty Factors used in MRL derivation:

[X] 3 for use of a minimal LOAEL
[X] 10 for extrapolation from animals to humans
[X] 10 for human variability

Was a conversion used from ppm in food or water to a mg/body weight dose? Not applicable.
If so, explain:

If an inhalation study in animals, list the conversion factors used in determining human equivalent dose:
Not applicable.

Other additional studies or pertinent information which lend support to this MRL: In humans, hepatic toxicity (fatty accumulation, necrosis) has been noted following ingestion of single doses of carbon tetrachloride in the range of 80–180 mg/kg (Docherty and Burgess 1922; Docherty and Nicholls 1923; Phelps and Hu 1924). Single doses of 70 mg/kg had no overt neurological effect, but various neurological symptoms indicative of depression of the central nervous system have been reported at doses between 114 and 10,800 mg/kg (Cohen 1957; Hall 1921; Leach 1922; Stevens and Forster 1953; Stewart et al. 1963). Gastrointestinal effects in humans following ingestion of single doses include nausea at ≥100 mg/kg (Ruprah et al. 1985) and vomiting and abdominal pain at 680–910 mg/kg (Hardin 1954; New et al. 1962; Smetana 1939; Umiker and Pearce 1953; von Oettingen 1964). In laboratory animals, mild hepatic effects (cytoplasmic vacuolization and increased serum enzymes) have been reported to occur following treatment with single doses of 40-80 mg/kg or repeated dosing at 5–20 mg/kg/day (Bruckner et al. 1986; Kim et al. 1990b; Korsrud et al. 1972; Smialowicz et al. 1991). No renal effects or positive results in special tests for immunological function were observed in rats following repeated administration at 5–160 mg/kg/day (Bruckner et al. 1986; Smialowicz et al. 1991). Renal effects (fatty degeneration, swelling of convoluted tubules) were observed in dogs given single doses of 3,200–6,400 mg/kg (Chandler and Chopra 1926; Gardner et al. 1925). Hepatic toxicity is selected as the critical effect of acute-duration oral exposure to carbon tetrachloride because effects were observed at the lowest effect level. The study of Smialowicz et al. (1991) is selected as the principal study because it provides the lowest LOAEL of 5 mg/kg/day for the critical effect.

Agency Contacts (Chemical Managers): Obaid Faroon, Ph.D.; Jessilynn Taylor, M.S.; Nickolette Roney, M.P.H.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Carbon Tetrachloride
CAS Number: 56-23-5
Date: June 2005
Profile Status: Final Post-Public Comment Draft
Route: [ ] Inhalation [X] Oral
Duration: [ ] Acute [X] Intermediate [ ] Chronic
Graph Key: 46
Species: Rat

Minimal Risk Level: 0.007 [X] mg/kg/day [ ] ppm

Reference: Bruckner JV, MacKenzi WF, Muralidhara S, et al. 1986. Oral toxicity of carbon tetrachloride: Acute, subacute and subchronic studies in rats. Fundam Appl Toxicol 6:16–34.

Experimental design: Male Sprague-Dawley rats (15–16/dose) were administered carbon tetrachloride (0, 1, 10, or 33 mg/kg) in corn oil by gavage 5 days/week for 12 weeks. Body weight was monitored twice weekly. Blood samples were collected from five rats per group just before dosing at 2, 4, 6, 8, 10, and 12 weeks for measurement of serum levels of sorbitol dehydrogenase (SDH), ornithine carbamyl transferase (OCT), alanine aminotransferase (ALT), and blood urea nitrogen (BUN). Each rat served as a blood donor twice during the study at 6-week intervals. At the end of the 12-week period, 7–9 rats per group were sacrificed and the remaining were maintained for 13 days without dosing before sacrifice. Histopathological examination of the liver and kidneys was performed.

Effects noted in study and corresponding doses: No adverse effects were noted at 1 mg/kg. Body weight gain was reduced in the 33 mg/kg group by 17% compared to controls after 90 days ($p<0.05$). At 10 mg/kg, there were statistically significant increases in serum SDH activity, 2-fold higher than controls, observed as early as 10 weeks; a statistically significant 35% elevation in serum ALT was observed in this group at 12 weeks. Elevations in these serum parameters returned to control levels during the recovery period. Mild centrilobular vacuolization was observed in the liver of all animals treated at 10 mg/kg/day for 12 weeks. Substantial liver toxicity was observed at 33 mg/kg as early as 2 weeks. At 2 weeks, serum ALT was elevated 5-fold, OCT 6-fold, and SDH 38-fold compared to controls; after 12 weeks, serum ALT was elevated 20-fold, OCT 5-fold, and SDH 45-fold compared to controls. Only the serum ALT elevation (2-fold) was still statistically different from controls after 2 weeks of recovery. The liver:body weight ratio was significantly elevated by 46% in the 33 mg/kg group compared to controls. Extensive hepatic lesions observed in the 33 mg/kg group after 12 weeks included vacuolization, periportal fibrosis, bile duct hyperplasia, hyperplastic nodules, and single-cell necrosis. Treatment with carbon tetrachloride had no significant effect on kidney:body weight ratios, a kidney-related serum parameter (BUN) or on the incidence of kidney lesions.

Dose and end point used for MRL derivation: The NOAEL of 1 mg/kg for mild centrilobular vacuolization and increased serum SDH was used to derive the MRL. The NOAEL was adjusted for intermittent exposure (5 days/7 days), resulting in a duration-adjusted NOAEL of 0.71 mg/kg/day.

[X] NOAEL [ ] LOAEL

Uncertainty Factors used in MRL derivation:

[ ] 10 for use of a LOAEL
[X] 10 for extrapolation from animals to humans
[X] 10 for human variability

Was a conversion used from ppm in food or water to a mg/body weight dose? Not applicable.
If so, explain:

If an inhalation study in animals, list the conversion factors used in determining human equivalent dose:
Not applicable.

Other additional studies or pertinent information which lend support to this MRL: The intermediate-duration oral toxicity database for carbon tetrachloride is somewhat limited in that no human data are available and many studies in laboratory animals restricted analysis to the liver or to the liver and kidney. The incidence and severity of hepatic effects were dose-related in animal studies. Whereas no hepatic effects were noted at 1 mg/kg, significantly elevated sorbitol dehydrogenase (SDH) and mild centrilobular vacuolization were noted in rats exposed at 10 mg/kg 5 days/week for 12 weeks (Bruckner et al. 1986). In mice ingesting carbon tetrachloride 5 days/week for 12–13 weeks, no hepatic effects were detected at a dose of 1.2 mg/kg (Condie et al. 1986). Significant elevation in some serum enzymes (ALT, aspartate aminotransferase [AST], lactate dehydrogenase [LDH]), and mild necrosis were seen in mice at doses of 12 mg/kg and higher (Condie et al. 1986; Hayes et al. 1986). More extensive hepatic lesions (fatty accumulation, fibrosis, cirrhosis, necrosis) were noted in rats at doses of 20–25 mg/kg and higher (Allis et al. 1990; Bruckner et al. 1986; Koporec et al. 1995). At 100 mg/kg/day, hepatic effects in rats also included cytomegaly and various types of hyperplasia, which were perhaps adaptive responses to necrosis (Koporec et al. 1995). Effects in other organ systems include reduced body weight gain at doses between 33 and 100 mg/kg/day (Bruckner et al. 1986; Koporec et al. 1995) and neurological effects (increased serotonin synthesis) at 290 mg/kg/day (Bengtsson et al. 1987). No renal effects were observed in mice exposed at 1,200 mg/kg/day despite hepatic effects at lower levels (Hayes et al. 1986). Increased mortality was observed in rats exposed at 25 mg/kg/day (Koporec et al. 1995) and cancer (hepatoma) in mice treated with 20 mg/kg/day for 120 days and hamsters treated once weekly with 120 mg/kg/day for 30 weeks (Eschenbrenner and Miller 1946; Della Porta et al. 1961). Hepatic effects were selected as the critical effects of intermediate-duration oral exposure to carbon tetrachloride because they occurred at the lowest effect level. The rat study of Bruckner was selected as the principal study because it provided the lowest LOAEL for the critical effect.

Agency Contacts (Chemical Managers): Obaid Faroon, Ph.D.; Jessilynn Taylor, M.S.; Nickolette Roney, M.P.H.

APPENDIX B. USER'S GUIDE

Chapter 1

Public Health Statement

This chapter of the profile is a health effects summary written in non-technical language. Its intended audience is the general public, especially people living in the vicinity of a hazardous waste site or chemical release. If the Public Health Statement were removed from the rest of the document, it would still communicate to the lay public essential information about the chemical.

The major headings in the Public Health Statement are useful to find specific topics of concern. The topics are written in a question and answer format. The answer to each question includes a sentence that will direct the reader to chapters in the profile that will provide more information on the given topic.

Chapter 2

Relevance to Public Health

This chapter provides a health effects summary based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information. This summary is designed to present interpretive, weight-of-evidence discussions for human health end points by addressing the following questions:

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

The chapter covers end points in the same order that they appear within the Discussion of Health Effects by Route of Exposure section, by route (inhalation, oral, and dermal) and within route by effect. Human data are presented first, then animal data. Both are organized by duration (acute, intermediate, chronic). *In vitro* data and data from parenteral routes (intramuscular, intravenous, subcutaneous, etc.) are also considered in this chapter.

The carcinogenic potential of the profiled substance is qualitatively evaluated, when appropriate, using existing toxicokinetic, genotoxic, and carcinogenic data. ATSDR does not currently assess cancer potency or perform cancer risk assessments. Minimal Risk Levels (MRLs) for noncancer end points (if derived) and the end points from which they were derived are indicated and discussed.

Limitations to existing scientific literature that prevent a satisfactory evaluation of the relevance to public health are identified in the Chapter 3 Data Needs section.

Interpretation of Minimal Risk Levels

Where sufficient toxicologic information is available, ATSDR has derived MRLs for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action, but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans.

MRLs should help physicians and public health officials determine the safety of a community living near a chemical emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Chapter 2, "Relevance to Public Health," contains basic information known about the substance. Other sections such as Chapter 3 Section 3.9, "Interactions with Other Substances," and Section 3.10, "Populations that are Unusually Susceptible" provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology that the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the most sensitive end point which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen end point are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest no-observed-adverse-effect level (NOAEL) that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor (UF) of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a substance-specific MRL are provided in the footnotes of the levels of significant exposure (LSE) tables.

Chapter 3

Health Effects

Tables and Figures for Levels of Significant Exposure (LSE)

Tables and figures are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species, MRLs to humans for noncancer end points, and EPA's estimated range associated with an upper- bound individual lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. Use the LSE tables and figures for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of NOAELs, LOAELs, or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE Table 3-1 and Figure 3-1 are shown. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

LEGEND

See Sample LSE Table 3-1 (page B-6)

(1) Route of Exposure. One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. Typically when sufficient data exist, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure, i.e., inhalation, oral, and dermal (LSE Tables 3-1, 3-2, and 3-3, respectively). LSE figures are limited to the inhalation (LSE Figure 3-1) and oral (LSE Figure 3-2) routes. Not all substances will have data on each route of exposure and will not, therefore, have all five of the tables and figures.

(2) Exposure Period. Three exposure periods—acute (less than 15 days), intermediate (15–364 days), and chronic (365 days or more)—are presented within each relevant route of exposure. In this example, an inhalation study of intermediate exposure duration is reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Health Effect. The major categories of health effects included in LSE tables and figures are death, systemic, immunological, neurological, developmental, reproductive, and cancer. NOAELs and LOAELs can be reported in the tables and figures for all effects but cancer. Systemic effects are further defined in the "System" column of the LSE table (see key number 18).

(4) Key to Figure. Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 18 has been used to derive a NOAEL and a Less Serious LOAEL (also see the two "18r" data points in sample Figure 3-1).

(5) Species. The test species, whether animal or human, are identified in this column. Chapter 2, "Relevance to Public Health," covers the relevance of animal data to human toxicity and Section 3.4, "Toxicokinetics," contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(6) Exposure Frequency/Duration. The duration of the study and the weekly and daily exposure regimens are provided in this column. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 18), rats were exposed to “Chemical x” via inhalation for 6 hours/day, 5 days/week, for 13 weeks. For a more complete review of the dosing regimen, refer to the appropriate sections of the text or the original reference paper (i.e., Nitschke et al. 1981).

(7) System. This column further defines the systemic effects. These systems include respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, and dermal/ocular. "Other" refers to any systemic effect (e.g., a decrease in body weight) not covered in these systems. In the example of key number 18, one systemic effect (respiratory) was investigated.

(8) NOAEL. A NOAEL is the highest exposure level at which no harmful effects were seen in the organ system studied. Key number 18 reports a NOAEL of 3 ppm for the respiratory system, which was used to derive an intermediate exposure, inhalation MRL of 0.005 ppm (see footnote "b").

(9) LOAEL. A LOAEL is the lowest dose used in the study that caused a harmful health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific end point used to quantify the adverse effect accompanies the LOAEL. The respiratory effect reported in key number 18 (hyperplasia) is a Less Serious LOAEL of 10 ppm. MRLs are not derived from Serious LOAELs.

(10) Reference. The complete reference citation is given in Chapter 9 of the profile.

(11) CEL. A CEL is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases.

(12) Footnotes. Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. Footnote "b" indicates that the NOAEL of 3 ppm in key number 18 was used to derive an MRL of 0.005 ppm.

LEGEND

See Sample Figure 3-1 (page B-7)

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(13) Exposure Period. The same exposure periods appear as in the LSE table. In this example, health effects observed within the acute and intermediate exposure periods are illustrated.

(14) Health Effect. These are the categories of health effects for which reliable quantitative data exists. The same health effects appear in the LSE table.

(15) Levels of Exposure. Concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(16) NOAEL. In this example, the open circle designated 18r identifies a NOAEL critical end point in the rat upon which an intermediate inhalation exposure MRL is based. The key number 18 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 3 ppm (see entry 18 in the table) to the MRL of 0.005 ppm (see footnote "b" in the LSE table).

(17) CEL. Key number 38m is one of three studies for which CELs were derived. The diamond symbol refers to a CEL for the test species-mouse. The number 38 corresponds to the entry in the LSE table.

(18) Estimated Upper-Bound Human Cancer Risk Levels. This is the range associated with the upper-bound for lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. These risk levels are derived from the EPA's Human Health Assessment Group's upper-bound estimates of the slope of the cancer dose response curve at low dose levels (q_1*).

(19) Key to LSE Figure. The Key explains the abbreviations and symbols used in the figure.

SAMPLE

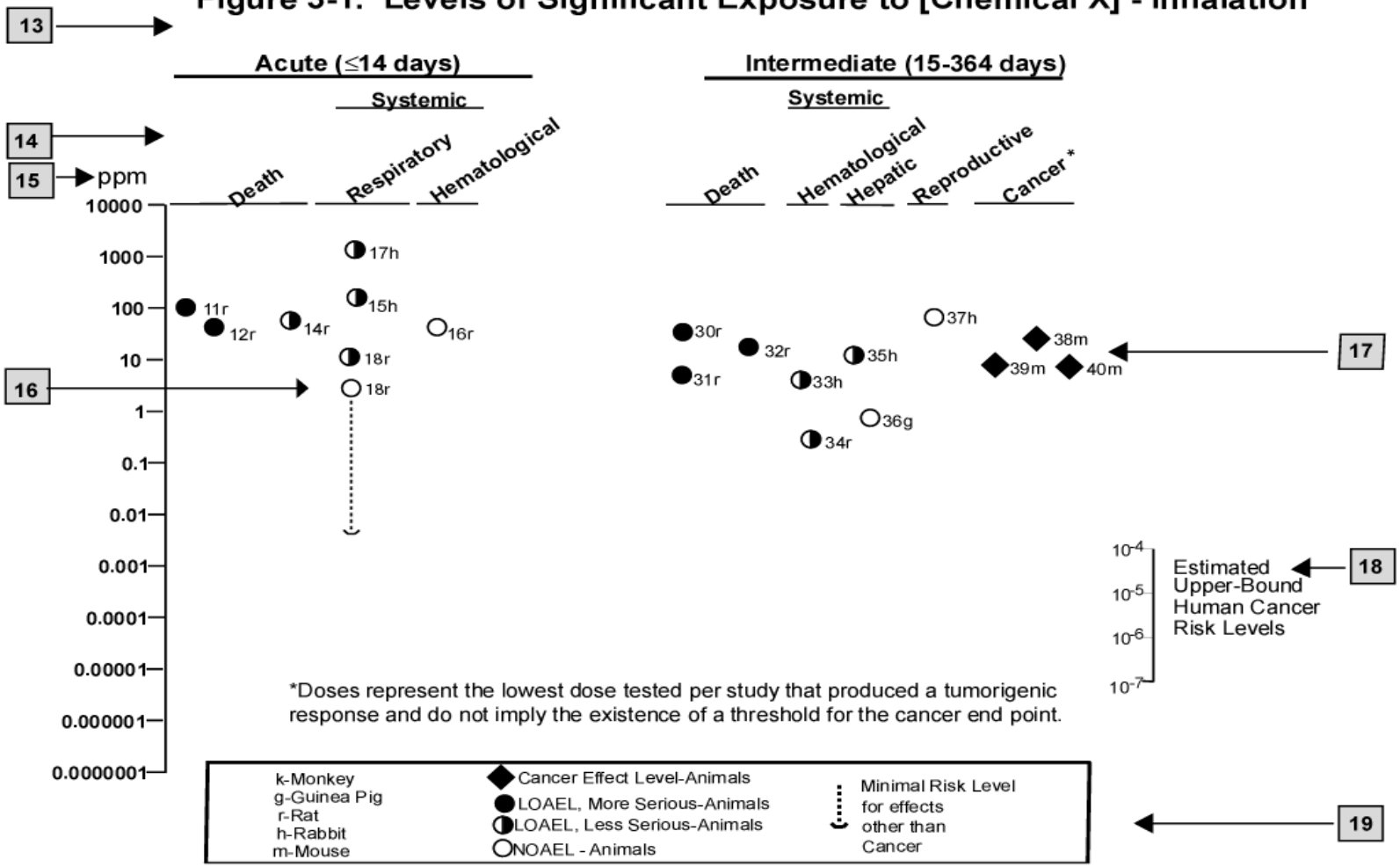
1 → **Table 3-1. Levels of Significant Exposure to [Chemical x] – Inhalation**

	Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
2 →	INTERMEDIATE EXPOSURE							
		5	6	7	8	9		10
3 →	Systemic	↓	↓	↓	↓	↓		↓
4 →	18	Rat	13 wk 5 d/wk 6 hr/d	Resp	3[b]	10 (hyperplasia)		Nitschke et al. 1981
	CHRONIC EXPOSURE							
	Cancer						11	
							↓	
	38	Rat	18 mo 5 d/wk 7 hr/d				20 (CEL, multiple organs)	Wong et al. 1982
	39	Rat	89–104 wk 5 d/wk 6 hr/d				10 (CEL, lung tumors, nasal tumors)	NTP 1982
	40	Mouse	79–103 wk 5 d/wk 6 hr/d				10 (CEL, lung tumors, hemangiosarcomas)	NTP 1982

12 → [a] The number corresponds to entries in Figure 3-1.
[b] Used to derive an intermediate inhalation Minimal Risk Level (MRL) of 5×10^{-3} ppm; dose adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animal to humans, 10 for human variability).

SAMPLE

Figure 3-1. Levels of Significant Exposure to [Chemical X] - Inhalation



APPENDIX C. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
ACOEM	American College of Occupational and Environmental Medicine
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
AED	atomic emission detection
AFID	alkali flame ionization detector
AFOSH	Air Force Office of Safety and Health
ALT	alanine aminotransferase
AML	acute myeloid leukemia
AOAC	Association of Official Analytical Chemists
AOEC	Association of Occupational and Environmental Clinics
AP	alkaline phosphatase
APHA	American Public Health Association
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AWQC	Ambient Water Quality Criteria
BAT	best available technology
BCF	bioconcentration factor
BEI	Biological Exposure Index
BMD	benchmark dose
BMR	benchmark response
BSC	Board of Scientific Counselors
C	centigrade
CAA	Clean Air Act
CAG	Cancer Assessment Group of the U.S. Environmental Protection Agency
CAS	Chemical Abstract Services
CDC	Centers for Disease Control and Prevention
CEL	cancer effect level
CELDS	Computer-Environmental Legislative Data System
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
Ci	curie
CI	confidence interval
CL	ceiling limit value
CLP	Contract Laboratory Program
cm	centimeter
CML	chronic myeloid leukemia
CPSC	Consumer Products Safety Commission
CWA	Clean Water Act
DHEW	Department of Health, Education, and Welfare
DHHS	Department of Health and Human Services
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	Department of Energy
DOL	Department of Labor
DOT	Department of Transportation

DOT/UN/NA/IMCO	Department of Transportation/United Nations/North America/International Maritime Dangerous Goods Code
DWEL	drinking water exposure level
ECD	electron capture detection
ECG/EKG	electrocardiogram
EEG	electroencephalogram
EEGL	Emergency Exposure Guidance Level
EPA	Environmental Protection Agency
F	Fahrenheit
F_1	first-filial generation
FAO	Food and Agricultural Organization of the United Nations
FDA	Food and Drug Administration
FEMA	Federal Emergency Management Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FPD	flame photometric detection
fpm	feet per minute
FR	Federal Register
FSH	follicle stimulating hormone
g	gram
GC	gas chromatography
gd	gestational day
GLC	gas liquid chromatography
GPC	gel permeation chromatography
HPLC	high-performance liquid chromatography
HRGC	high resolution gas chromatography
HSDB	Hazardous Substance Data Bank
IARC	International Agency for Research on Cancer
IDLH	immediately dangerous to life and health
ILO	International Labor Organization
IRIS	Integrated Risk Information System
Kd	adsorption ratio
kg	kilogram
kkg	metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{50}	lethal concentration, 50% kill
LC_{Lo}	lethal concentration, low
LD_{50}	lethal dose, 50% kill
LD_{Lo}	lethal dose, low
LDH	lactic dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
LT_{50}	lethal time, 50% kill
m	meter
MA	*trans,trans*-muconic acid
MAL	maximum allowable level
mCi	millicurie
MCL	maximum contaminant level

MCLG	maximum contaminant level goal
MF	modifying factor
MFO	mixed function oxidase
mg	milligram
mL	milliliter
mm	millimeter
mmHg	millimeters of mercury
mmol	millimole
mppcf	millions of particles per cubic foot
MRL	Minimal Risk Level
MS	mass spectrometry
NAAQS	National Ambient Air Quality Standard
NAS	National Academy of Science
NATICH	National Air Toxics Information Clearinghouse
NATO	North Atlantic Treaty Organization
NCE	normochromatic erythrocytes
NCEH	National Center for Environmental Health
NCI	National Cancer Institute
ND	not detected
NFPA	National Fire Protection Association
ng	nanogram
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NIOSHTIC	NIOSH's Computerized Information Retrieval System
NLM	National Library of Medicine
nm	nanometer
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NOES	National Occupational Exposure Survey
NOHS	National Occupational Hazard Survey
NPD	nitrogen phosphorus detection
NPDES	National Pollutant Discharge Elimination System
NPL	National Priorities List
NR	not reported
NRC	National Research Council
NS	not specified
NSPS	New Source Performance Standards
NTIS	National Technical Information Service
NTP	National Toxicology Program
ODW	Office of Drinking Water, EPA
OERR	Office of Emergency and Remedial Response, EPA
OHM/TADS	Oil and Hazardous Materials/Technical Assistance Data System
OPP	Office of Pesticide Programs, EPA
OPPT	Office of Pollution Prevention and Toxics, EPA
OPPTS	Office of Prevention, Pesticides and Toxic Substances, EPA
OR	odds ratio
OSHA	Occupational Safety and Health Administration
OSW	Office of Solid Waste, EPA
OTS	Office of Toxic Substances
OW	Office of Water

OWRS	Office of Water Regulations and Standards, EPA
PAH	polycyclic aromatic hydrocarbon
PBPD	physiologically based pharmacodynamic
PBPK	physiologically based pharmacokinetic
PCE	polychromatic erythrocytes
PEL	permissible exposure limit
pg	picogram
PHS	Public Health Service
PID	photo ionization detector
pmol	picomole
PMR	proportionate mortality ratio
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
PSNS	pretreatment standards for new sources
RBC	red blood cell
REL	recommended exposure level/limit
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
RQ	reportable quantity
RTECS	Registry of Toxic Effects of Chemical Substances
SARA	Superfund Amendments and Reauthorization Act
SCE	sister chromatid exchange
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
SIC	standard industrial classification
SIM	selected ion monitoring
SMCL	secondary maximum contaminant level
SMR	standardized mortality ratio
SNARL	suggested no adverse response level
SPEGL	Short-Term Public Emergency Guidance Level
STEL	short term exposure limit
STORET	Storage and Retrieval
TD_{50}	toxic dose, 50% specific toxic effect
TLV	threshold limit value
TOC	total organic carbon
TPQ	threshold planning quantity
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TWA	time-weighted average
UF	uncertainty factor
U.S.	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey
VOC	volatile organic compound
WBC	white blood cell
WHO	World Health Organization

>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to
%	percent
α	alpha
β	beta
γ	gamma
δ	delta
µm	micrometer
µg	microgram
q_1^*	cancer slope factor
–	negative
+	positive
(+)	weakly positive result
(–)	weakly negative result

APPENDIX D. INDEX

absorbed dose 103, 104, 114, 116, 122, 136, 137, 190
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TOXICOLOGICAL PROFILE FOR CHLOROBENZENE

Agency for Toxic Substances and Disease Registry
U.S. Public Health Service

December 1990

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The use of company or product name(s) is for identification only and does not imply endorsement by the Agency for Toxic Substances and Disease Registry.

FOREWORD

The Superfund Amendments and Reauthorization Act (SARA) of 1986 (Public Law 99-499) extended and amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). This public law directed the Agency for Toxic Substances and Disease Registry (ATSDR) to prepare toxicological profiles for hazardous substances which are most commonly found at facilities on the CERCLA National Priorities List and which pose the most significant potential threat to human health, as determined by ATSDR and the Environmental Protection Agency (EPA). The lists of the 250 most significant hazardous substances were published in the Federal Register on April 17, 1987, on October 20, 1988, on October 26, 1989, and on October 17, 1990.

Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list. Each profile must include the following content:

(A) An examination, summary, and interpretation of available toxicological information and epidemiological evaluations on the hazardous substance in order to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects,

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure which present a significant risk to human health of acute, subacute, and chronic health effects, and

(C) Where appropriate, an identification of toxicological testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

This toxicological profile is prepared in accordance with guidelines developed by ATSDR and EPA. The original guidelines were published in the Federal Register on April 17, 1987. Each profile will be revised and republished as necessary, but no less often than every three years, as required by CERCLA, as amended.

The ATSDR toxicological profile is intended to characterize succinctly the toxicological and adverse health effects information for the hazardous substance being described. Each profile identifies and reviews the key literature (that has been peer-reviewed) that describes a hazardous substance's toxicological properties. Other pertinent literature is also presented but described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

Foreword

Each toxicological profile begins with a public health statement, which describes in nontechnical language a substance's relevant toxicological properties. Following the public health statement is information concerning significant health effects associated with exposure to the substance. The adequacy of information to determine a substance's health effects is described. Data needs that are of significance to protection of public health will be identified by ATSDR, the National Toxicology Program (NTP) of the Public Health Service, and EPA. The focus of the profiles is on health and toxicological information; therefore, we have included this information in the beginning of the document.

The principal audiences for the toxicological profiles are health professionals at the federal, state, and local levels, interested private sector organizations and groups, and members of the public.

This profile reflects our assessment of all relevant toxicological testing and information that has been peer reviewed. It has been reviewed by scientists from ATSDR, the Centers for Disease Control, the NTP, and other federal agencies. It has also been reviewed by a panel of nongovernment peer reviewers and is being made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

William L. Roper, M.D., M.P.H.
Administrator
Agency for Toxic Substances and
Disease Registry

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1. PUBLIC HEALTH STATEMENT

This Statement was prepared to give you information about chlorobenzene and to emphasize the human health effects that may result from exposure to it. The Environmental Protection Agency (EPA) has identified 1,177 sites on its National Priorities List (NPL). Chlorobenzene has been found at 97 of these sites. However, we do not know how many of the 1,177 NPL sites have been evaluated for chlorobenzene. As EPA evaluates more sites, the number of sites at which chlorobenzene is found may change. The information is important for you because chlorobenzene may cause harmful health effects and because these sites are potential or actual sources of human exposure to chlorobenzene.

When a chemical is released from a large area, such as an industrial plant, or from a container, such as a drum or bottle, it enters the environment as a chemical emission. This emission, which is also called a release, does not always lead to exposure. You can be exposed to a chemical only when you come into contact with the chemical. You may be exposed to it in the environment by breathing, eating, or drinking substances containing the chemical or from skin contact with it.

If you are exposed to a hazardous substance such as chlorobenzene, several factors will determine whether harmful health effects will occur and what the type and severity of those health effects will be. These factors include the dose (how much), the duration (how long), the route or pathway by which you are exposed (breathing, eating, drinking, or skin contact), the other chemicals to which you are exposed, and your individual characteristics such as age, sex, nutritional status, family traits, life style, and state of health.

1.1 WHAT IS CHLOROBENZENE?

Chlorobenzene is a colorless liquid with an almond-like odor. The compound does not occur widely in nature, but is manufactured for use as a solvent (a substance used to dissolve other substances) and is used in the production of other chemicals. Chlorobenzene persists in soil (several months), in air (3.5 days), and water (less than 1 day). Additional information can be found in Chapters 3, 4, and 5.

1.2 HOW MIGHT I BE EXPOSED TO CHLOROBENZENE?

There is potential for humans to be exposed to chlorobenzene by breathing contaminated air, by drinking water or eating food

contaminated with chlorobenzene, or by getting chlorobenzene-contaminated soil on the skin. These exposures are most likely to occur in the workplace or in the vicinity of chemical waste sites.

Occupational exposure occurs primarily through breathing the chemical. Personnel engaged in the production and handling of chlorobenzene would be at greatest risk. Levels of chlorobenzene in the air at several industrial sites during normal operations were found to be below allowable federal standards.

Exposure in humans could occur in persons living or working in the vicinity of hazardous waste sites if emissions to water, air, and soil are not adequately controlled. Chlorobenzene has been found at 97 out of 1,177 NPL hazardous waste sites in the United States. Thus, federal and state surveys suggest that chlorobenzene is not a widespread environmental contaminant. The chemical has not been detected in surface water, although a few ground water systems have been found with chlorobenzene levels in the parts per billion (ppb) range. Background levels of less than 1 ppb were detected in air samples from urban and suburban areas. No information of the occurrence of chlorobenzene in food has been found. Additional information on the potential for human exposure is presented in Chapter 5.

1.3 HOW CAN CHLOROBENZENE ENTER AND LEAVE MY BODY?

Chlorobenzene enters your body when you breathe in air containing it, when you drink water or eat food containing it, or when it comes in contact with your skin. Human exposure to contaminated water could occur near hazardous waste sites where chlorobenzene is present. Significant exposure to chlorobenzene is not expected to occur by getting chlorobenzene contaminated soil on your skin. When chlorobenzene enters your body, most of it is expelled from your lungs in the air we breathe out and in urine. Additional information is presented in Chapter 2.

1.4 HOW CAN CHLOROBENZENE AFFECT MY HEALTH?

Workers exposed to high levels of chlorobenzene complained of headaches, numbness, sleepiness, nausea, and vomiting. However, it is not known if chlorobenzene alone was responsible for these health effects since the workers may have also been exposed to other chemicals at the same time. Mild to severe depression of functions of parts of the nervous system is a common response to exposure to a wide variety of industrial solvents (a substance that dissolves other substances).

In animals, exposure to high concentrations of chlorobenzene affects the brain, liver, and kidneys. Unconsciousness, tremors and restlessness have been observed. The chemical can cause severe injury

to the liver and kidneys. Data indicate that chlorobenzene does not affect reproduction or cause birth defects. Studies in animals have shown that chlorobenzene can produce liver nodules, providing some but not clear evidence of cancer risk. Additional information on health effects is presented in Chapter 2.

1.5 WHAT LEVELS OF EXPOSURE HAVE RESULTED IN HARMFUL HEALTH EFFECTS?

Harm to human health from breathing, eating or drinking chlorobenzene is not established (Tables l-l and l-3). Tables l-2 and l-4 show the relationship between exposure to chlorobenzene and known health effects in animals. A Minimal Risk Level (MRL) is included in Table l-3. The MRL was derived from animal data for long-term exposure, as described in Chapter 2 and in Table 2-2. The MRL provides a basis for comparison with levels that people might encounter either in the air or in food or drinking water. If a person is exposed to chlorobenzene at an amount below the MRL, it is not expected that harmful (noncancer) health effects will occur. Because this level is based only on information currently available, some uncertainty is always associated with it. Also, because the method for deriving MRLs does not use any information about cancer, a MRL does not imply anything about the presence, absence, or level of risk for cancer. Further information on the levels of chlorobenzene that have been observed to cause health effects in animals is presented in Chapter 2.

1.6 IS THERE A MEDICAL TEST TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO CHLOROBENZENE?

Exposure to chlorobenzene can be determined by measuring the chemical or its metabolite in urine, exhaled air, blood, and body fat. Tests are not routinely available at the doctor's office. Specific tests are available that can determine if exposure is currently occurring or has occurred very recently, but not whether exposure occurred in the past. Further, levels in the various media stated above do not predict adverse health effects. Additional information on how chlorobenzene can be measured in exposed humans is given in Chapters 2 and 6.

1. PUBLIC HEALTH STATEMENT

TABLE 1-1. Human Health Effects from Breathing Chlorobenzene*

Short-term Exposure (less than or equal to 14 days)		
Levels in Air	Length of Exposure	Description of Effects
		The health effects resulting from short-term exposure of humans to air containing specific levels of chlorobenzene are not known.
Long-term Exposure (greater than 14 days)		
Levels in Air	Length of Exposure	Description of Effects
		The health effects resulting from long-term exposure of humans to air containing specific levels of chlorobenzene are not known.

*See Section 1.2 for a discussion of exposures encountered in daily life.

TABLE 1-2. Animal Health Effects from Breathing Chlorobenzene

Short-term Exposure (less than or equal to 14 days)		
Levels in Air (ppm)	Length of Exposure	Description of Effects*
537	2 hours	Death in rabbits.
Long-term Exposure (greater than 14 days)		
Levels in Air (ppm)	Length of Exposure	Description of Effects*
75	24 weeks	Liver and kidney damage in rats and rabbits.

*These effects are listed at the lowest level at which they were first observed. They may also be seen at higher levels.

TABLE 1-3. Human Health Effects from Eating or Drinking Chlorobenzene

Short-term Exposure (less than or equal to 14 days)		
Levels in Food	Length of Exposure	Description of Effects
		The health effects resulting from short-term exposure of humans to food containing specific levels of chlorobenzene are not known.
Levels in Water		The health effects resulting from short-term exposure of humans to water containing specific levels of chlorobenzene are not known.
Long-term Exposure (greater than 14 days)		
Levels in Food (ppm)	Length of Exposure	Description of Effects
15	91 days	Minimal Risk Level (based *on animal studies; see* Section 1.5 for discussion).
Levels in Water		The health effects resulting from long-term exposure of animals to water containing specific levels of chlorobenzene are not known.

TABLE 1-4. Animal Health Effects from Eating or Drinking Chlorobenzene

Short-term Exposure (less than or equal to 14 days)		
Levels in Food (ppm)	Length of Exposure	Description of Effects*
7,692 - 20,000	1-14 days	Death in mice and rats.
Levels in Water		
		The health effects of short-term exposure of animals to water containing specific levels of chlorobenzene are not known.
Long-term Exposure (greater than 14 days)		
Levels in Food (ppm)	Length of Exposure	Description of Effects*
1,923 - 5,000	91 days	Liver and kidney damage in mice. Liver injury rats.
1,923	13 weeks	Injury to organs of the immune system in mice.
1,923	13 weeks	Death in mice.
Levels in Water		
		The health effects resulting from long-term exposure of animals to water containing specific levels of chlorobenzene are not known.

*These effects are listed at the lowest level at which they were first observed. They may also be seen at higher levels.

1.7 WHAT RECOMMENDATIONS HAS THE FEDERAL GOVERNMENT MADE TO PROTECT HUMAN HEALTH?

The Federal Government has developed regulatory standards and advisories to protect individuals from potential health effects of cholorobenzene in the environment. The Environmental Protection Agency has proposed that the maximum level of chlorobenzene in drinking water be 0.1 parts per million (ppm). For short-term exposures to drinking water, EPA has recommended that drinking water levels not exceed 2 ppm for up to ten days. The Occupational Safety and Health Administration (OSHA) has established a legally enforceable minimum limit of 75 ppm of chlorobenzene in workplace air for an 8 hour/day, 40-hour work week. Additional information regarding federal and state regulations is presented in Chapter 7.

1.8 WHERE CAN I GET MORE INFORMATION?

If you have any more questions or concerns not covered here, please contact your State Health or Environmental Department or:

Agency for Toxic Substance and Disease Registry
Division of Toxicology
1600 Clifton Road, E-29
Atlanta, Georgia 30333

This agency can also give you information on the location of the nearest occupational and environmental health clinics. Such clinics specialize in recognizing, evaluating, and treating illnesses that result from exposure to hazardous substances.

2. HEALTH EFFECTS

2.1 INTRODUCTION

This chapter contains descriptions and evaluations of studies and interpretation of data on the health effects associated with exposure to chlorobenzene. Its purpose is to present levels of significant exposure for chlorobenzene based on toxicological studies, epidemiological investigations, and environmental exposure data. This information is presented to provide public health officials, physicians, toxicologists, and other interested individuals and groups with (1) an overall perspective of the toxicology of chlorobenzene and (2) a depiction of significant exposure levels associated with various adverse health effects.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals address the needs of persons living or working near hazardous waste sites, the data in this section are organized first by route of exposure -- inhalation, oral, and dermal -- and then by health effect -- death, systemic, immunological, neurological, developmental, reproductive, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods -- acute, intermediate, and chronic.

Levels of significant exposure for each exposure route and duration (for which data exist) are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELS have been classified into "less serious" or "serious" effects. These distinctions are intended to help the users of the document identify the levels of exposure at which adverse health effects start to appear, determine whether or not the intensity of the effects varies with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown on the tables and graphs may differ depending on the user's perspective. For example, physicians concerned with the interpretation of clinical findings in exposed persons or with the identification of persons with the potential to develop such disease may be interested in levels of exposure associated with "serious effects". Public health officials and project managers concerned with response actions at Superfund sites may want information on levels of exposure associated with more subtle effects in

humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (minimal risk levels, MRLs) are of interest to health professionals and citizens alike.

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made, where data were believed reliable, for the most sensitive noncancer end point for each exposure duration. MRLs include adjustments to reflect human variability and, where appropriate, the uncertainty of extrapolating from laboratory animal data to humans. Although methods have been established to derive these levels (Barnes et al. 1987; EPA 1989a), uncertainties are associated with the techniques.

2.2.1 Inhalation Exposure

2.2.1.1 Death

No studies were located regarding lethality in humans following inhalation exposure to chlorobenzene.

The acute lethality of chlorobenzene is relatively low in animals. Exposure to concentrations of 20 mg/L (4,300 ppm) for 2 hours resulted in 100% mortality in mice (Rozenbaum et al. 1947). Rabbits died 2 weeks after chlorobenzene exposure to concentrations of about 537 ppm (Rozenbaum et al. 1947).

The highest NOAEL values and all reliable LOAEL values for death in each species and duration category are recorded in Table 2-1 and plotted in Figure 2-1.

2.2.1.2 Systemic Effects

No studies were located regarding effects on the respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, and dermal/ocular systems in humans following inhalation exposure to chlorobenzene.

As shown in Table 2-1 and Figure 2-1, animal studies indicate that chlorobenzene induces injury to the liver and kidneys following intermediate and chronic inhalation exposures.

TABLE 2-1. Levels of Significant Exposure to Chlorobenzene - Inhalation

Figure Key	Species	Exposure Frequency/ Duration	Effect	NOAEL (ppm)	LOAEL (Effect) Less Serious (ppm)	LOAEL (Effect) Serious (ppm)	Reference
ACUTE EXPOSURE							
Death							
1	Rabbit	2 hr				537[a]	Rozenbaum 1947
2	Mouse	2 hr				4300	Rozenbaum 1947
Developmental							
3	Rat	10 d Gd6-15 6hr/d		590			John et al. 1984
4	Rabbit	13 d Gd6-18 6hr/d		590			John et al. 1984
INTERMEDIATE EXPOSURE							
Systemic							
5	Rat	120 d 5d/wk 7hr/d	Renal		75[a] (micro. lesions)		Dilley 1977
6	Rat	120 d 5d/wk 7hr/d	Hepatic		75[a] (decr. SGOT)		Dilley 1977
7	Rabbit	120 d 5d/wk 7hr/d	Hepatic		75[a] (decr. LDH)		Dilley 1977

TABLE 2-1 (Continued)

Figure Key	Species	Exposure Frequency/ Duration	Effect	NOAEL (ppm)	LOAEL (Effect) Less Serious (ppm)	LOAEL (Effect) Serious (ppm)	Reference
CHRONIC EXPOSURE							
Systemic							
8	Rat	2 gen 7d/wk 6hr/d	Hepatic	50	150 (hypertrophy)		Nair et al. 1987
9	Rat	2 gen 7d/wk 6hr/d	Renal	50	150 (micro. changes)		Nair et al. 1987
Reproductive							
10	Rat	2 gen 7d/wk 6hr/d		450			Nair et al. 1987

[a]Presented in Table 1-2.

LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; ppm = parts per million; hr = hour; LC100 = lethal concentration, 100% animals exposed; d = day; Gd = gestation day; wk = week; decr = decrease; SGOT = serum glutamic oxaloacetic transaminase; micro = microscopic; LDH = lactate dehydrogenase; gen = generation

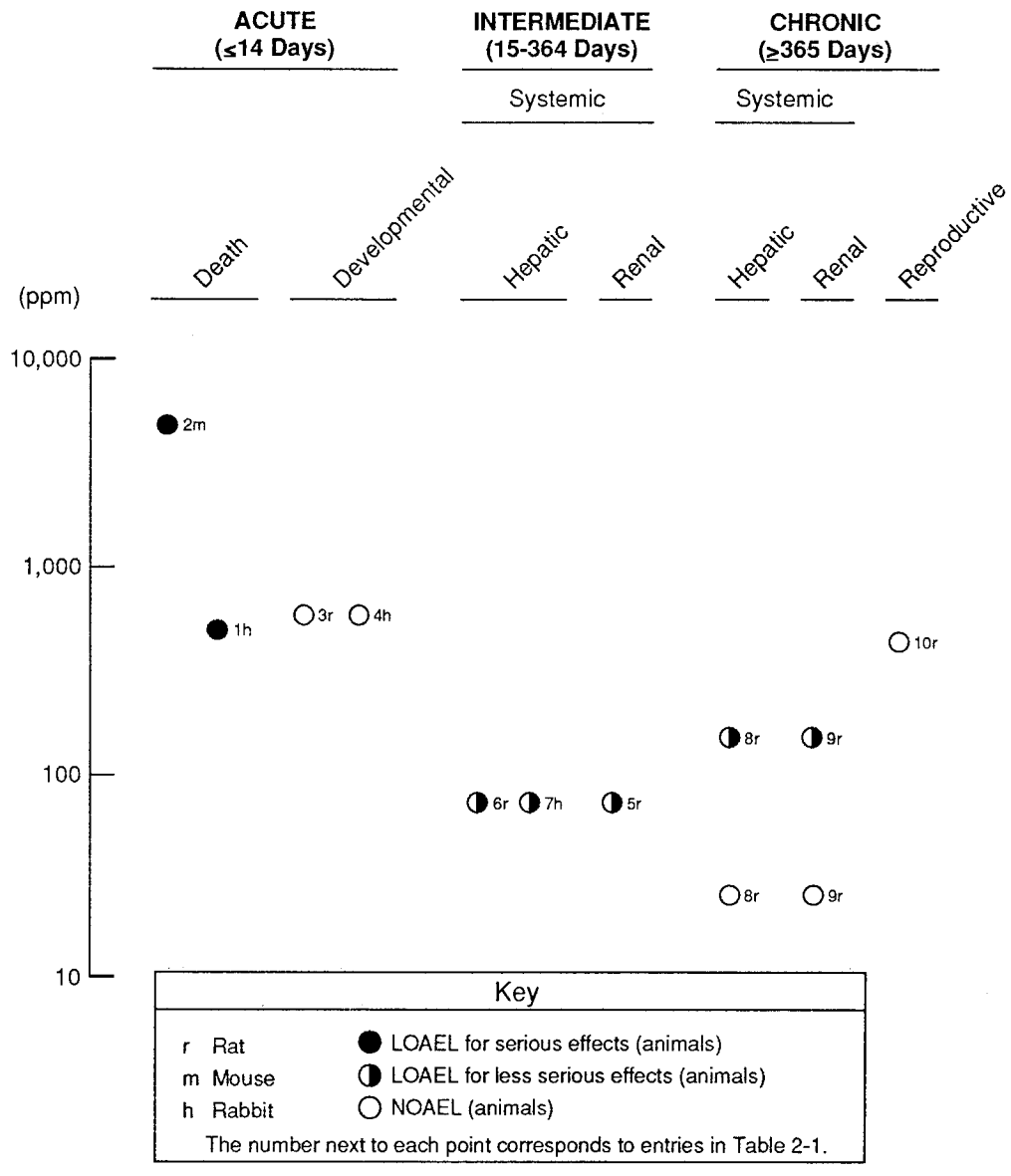


FIGURE 2-1. Levels of Significant Exposure to Chlorobenzene – Inhalation

Hematological. Based on a small number of studies, chlorobenzene may cause hematological changes. There were dose and time-related effects on red blood cell parameters, primarily an increase in reticulocyte count which increased in rats but not in rabbits exposed to vapors of chlorobenzene at concentrations ≥ 75 ppm for 24 weeks (Dilley 1977). Other hematological parameters (red blood cell count, hemoglobins, hematocrit, and white blood cell count) were variable and were comparable to controls at the end of the test. Slight leukopenia and lymphocytosis occurred in mice exposed to chlorobenzene (0.1 mg/L) for 3 months (Zub 1978). In the absence of more detailed experimental data and information on compound purity, it is not certain if the effects in mice were compound-related. Further, these effects have not been confirmed at comparable doses in other species. Thus, it appears that hematological effects may not be sensitive indicators of chlorobenzene toxicity.

Hepatic Effects. No data were found that severe liver damage results from acute exposure to chlorobenzene vapor. Treatment-related congestion of the liver was observed in male rats and to a lesser degree in male rabbits exposed for 24 weeks to ≥ 75 ppm (Dilley 1977). Focal hemorrhages and foci of perivascular lymphocytes were observed. Decreased levels of serum enzymes (lactate dehydrogenase [LDH] and serum glutamic-oxaloacetic transaminase [SGOT]) were observed at the end of the treatment period; the significance of this response is not clear. Nair et al. (1987) reported liver hypertrophy and increased liver weights in male rats exposed to chlorobenzene vapors daily at 150 and 450 ppm for two generations. Overall, data suggest liver toxicity may be an area of concern for chlorobenzene exposure in humans.

The highest NOAEL values and all reliable LOAEL values for liver toxicity in each species and duration category are recorded in Table 2-l and plotted in Figure 2-l.

Renal Effects. A small number of studies demonstrates that the kidney is also a target organ following chlorobenzene exposure and that the effects occur at levels comparable to those causing liver effects. Nair et al. (1987) reported tubular dilatation with eosinophilic material, interstitial nephritis and foci of regenerative epithelium in male rats exposed to vapors of chlorobenzene at 150 and 450 ppm for two generations. There was also treatment-related congestion of the kidneys in rabbits exposed to chlorobenzene at concentrations 275 ppm in animals sacrificed at 5 weeks of a 24 week treatment period (Dilley 1977). Interstitial foci of lymphocytes were evident. Overall, data suggest that this effect may also be an area of concern for chlorobenzene exposure in humans.

The highest NOAEL values and all reliable LOAEL values for renal toxicity in each species and duration category are recorded in Table 2-l and plotted in Figure 2-l.

2.2.1.3 Immunological Effects

No studies were located regarding the immunological effects in humans or animals following inhalation exposure to chlorobenzene.

2.2.1.4 Neurological Effects

Chlorobenzene affects the central nervous system. Humans occupationally exposed to chlorobenzene intermittently for up to 2 years at levels above current federal limits displayed signs of neurotoxicity including numbness, cyanosis (from depression of respiratory center), hyperesthesia, and muscle spasms (Rozenbaum et al. 1947). Specific exposure levels and histopathologic data have not been provided.

Neurological effects of chlorobenzene have also been reported in animals following inhalation. Acute inhalation exposure produced muscle spasms followed by narcosis in rabbits exposed to 5 mg/L chlorobenzene (1,090 ppm) or greater for 2 hours (Rozenbaum et al. 1947).

2.2.1.5 Developmental Effects

No studies were located regarding developmental effects in humans following inhalation exposure to chlorobenzene.

In rats and rabbits, inhalation of chlorobenzene vapors at concentrations up to 590 ppm during periods of major organogenesis did not produce structural malformations (John et al. 1984). This value has been presented in Table 2-l and plotted in Figure 2-l. The highest dose resulted in maternal toxicity, as indicated by elevation of liver weights (both species) and decreased food consumption and body weight gain (rats only).

2.2.1.6 Reproductive Effects

No studies were located regarding reproductive effects in humans following inhalation exposure to chlorobenzene.

In a two-generation study in rats, chlorobenzene in concentrations up to 450 ppm did not adversely affect reproductive performance or fertility (Nair et al. 1987). This value has been presented in Table 2-l and plotted in Figure 2-1. A slight increase was observed in the incidence of degenerative testicular changes (unilateral and bilateral) in high-dose (450 ppm) males (F_0 and F_1 generations) and the F_1 mid-dose (150 ppm) males. The significance of this finding is

unclear since the mean mating, pregnancy, and male fertility indices for both F_0 and F_1 generations were comparable for all groups and the incidences of testicular lesion were identical in F_0 and F_1 animals.

2.2.1.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or animals following inhalation exposure to chlorobenzene.

2.2.1.8 Cancer

No studies were located regarding carcinogenic effects in humans or animals following inhalation exposure to chlorobenzene.

2.2.2 Oral Exposure

2.2.2.1 Death

No studies were located regarding lethality in humans following oral exposure to chlorobenzene.

Animal studies show that chlorobenzene is lethal following acute, intermediate, and chronic oral exposures. Death occurred within 2 to 3 days after a single exposure to 4,000 mg/kg in corn oil by gavage in rats of both sexes, and in mice after a single exposure to 1,000 mg/kg (NTP 1985). Necropsy or histological examination was not performed. In a 14-day repeated-dose gavage study in rats, administration of $\geq$ 1,000 mg/kg was lethal to all rats by the end of the study (NTP 1985). This dose has been converted to an equivalent concentration of 20,000 ppm in food for presentation in Table 1-4. Survival was reduced in rats of both sexes exposed to $\geq$500 mg/kg/day and $\geq$250 mg/kg/day in mice following intermediate-duration exposure (NTP 1985). The dose of 250 mg/kg/day has been converted to an equivalent concentration of 1,923 ppm in food for presentation in Table l-4. Clinical signs of toxicity were not observed in mice and rats but histopathologic examination revealed dose-related chemical-induced changes to the liver, kidney, bone marrow, spleen, and thymus. Liver and kidney weights increased in mice and rats, while spleen weights decreased. In chronic oral studies, male rat survival at 120 mg/kg (2,400 ppm) was significantly lower than that of vehicle controls (NTP 1985); however no compound-induced toxic lesions responsible for this reduction in survival were observed.

The highest NOAEL values and all reliable LOAEL values for death in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.2 Systemic Effects

No studies in humans were located regarding the effects on the respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, and dermal/ocular systems following oral exposure to chlorobenzene. The following sections describe effects observed in animals.

Hepatic Effects. Animal studies indicate that the liver is susceptible to injury by chlorobenzene following oral exposure. Typical signs include: increased serum enzymes, altered liver weights, degeneration, necrosis, and interference with porphyrin metabolism. In acute studies (5 days), effects on porphyrin metabolism occurred at 1,140 mg/kg/day by gavage (Rimington and Ziegler 1963). Intermediate and long-term exposure studies in rats and mice reported organ weight increases at 100 (Hazleton 1967) and 125 mg/kg/day (NTP 1985), while organ weight increases and microscopic lesions were detected at ≥ 250 mg/kg/day by the same route (NTP 1985). Focal hepatocytic necrosis and degenerative changes in the centrilobular hepatocytes were observed in mice. These effects were most apparent in the ≥500 mg/kg dose group in rats. The dose of 250 mg/kg/day has been converted to equivalent concentrations of 1,923 ppm (in mice) and 5,000 ppm (in rats) in food for presentation in Table l-4. No effects were observed at 60 mg/kg/day. Based on this value, an intermediate oral MRL of 0.4 mg/kg/day was calculated as described in the footnote in Table 2-2. This MRL has been converted to an equivalent concentration in food (15 ppm) for presentation in Table 1-3.

Renal Effects. Animal studies demonstrate that chlorobenzene can cause injury to the kidney at doses comparable to those which cause liver effects. In a 90-day study, degeneration or focal necrosis of the proximal tubules was observed at ≥250 mg/kg in mice and ≥500 mg/kg in rats (NTP 1985). Repeated doses of ≥100 mg/kg/day for 90 to 99 days (Hazleton 1967) caused an increase in kidney weights.

TABLE 2-2. Levels of Significant Exposure to Chlorobenzene - Oral

Figure Key	Species	Route	Exposure Frequency/ Duration	Effect	NOAEL (mg/kg/day)	LOAEL (Effect) Less Serious (mg/kg/day)	LOAEL (Effect) Serious (mg/kg/day)	Reference
ACUTE EXPOSURE								
Death								
1	Rat	(G)	14 d 1x/d		500		1000[a]	NTP 1985
2	Rat	(G)	1 d 1x/d		250		4000	NTP 1985
3	Mouse	(G)	14 d 1x/d		500			NTP 1985
4	Mouse	(G)	1 d 1x/d				1000[b]	NTP 1985
Systemic								
5	Rat	(G)	5 d	Hepatic			1140 (necrosis)	Rimington and Ziegler 1963
Neurological								
6	Rat	(G)	1 d 1x/d				4000 (prostration)	NTP 1985
7	Rat	(G)	14 d 1x/d				1000 (prostration)	NTP 1985
INTERMEDIATE EXPOSURE								
Death								
8	Rat	(G)	91 d 5d/wk		250			NTP 1985
9	Mouse	(G)	91 d 5d/wk		125			NTP 1985
Systemic								
10	Rat	(G)	91 d 5d/wk	Hepatic	60[c]	125 (incr. wt. and serum enzymes)	250[d] (necrosis)	NTP 1985

TABLE 2-2 (Continued)

Figure Key	Species	Route	Exposure Frequency/ Duration	Effect	NOAEL (mg/kg/day)	LOAEL (Effect) Less Serious (mg/kg/day)	LOAEL (Effect) Serious (mg/kg/day)	Reference
11	Mouse	(G)	91 d 5d/wk	Renal	125		250[e] (necrosis, tub. degen.)	NTP 1985
12	Mouse	(G)	91 d 5d/wk	Hepatic	60	125 (incr. wt.)	250[e] (necrosis, degeneration)	NTP 1985
Immunological								
13	Mouse	(G)	91 d 5d/wk				250 (thymic necrosis, splenic depletion)	NTP 1985
CHRONIC EXPOSURE								
Death								
14	Mouse	(G)	103 wk 5d/wk 1x/d		120			NTP 1985
Systemic								
15	Rat	(G)	103 wk 5d/wk 1x/d	Hepatic	60		120 (necrosis)	NTP 1985

[a]Converted to an equivalent concentration of 20,000 ppm in food for presentation in Table 1-4.
[b]Converted to an equivalent concentration of 7692 ppm in food for presentation in Table 1-4.
[c]Used to derive intermediate oral MRL of 0.4 mg/kg/day; dose adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans, and 10 for human variability). This MRL has been converted to an equivalent concentration in food (15 ppm) for presentation in Table 1-3.
[d]Converted to an equivalent concentration of 5000 ppm in food for presentation in Table 1-4.
[e]Converted to an equivalent concentration of 1923 ppm in food for presentation in Table 1-4.

LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; mg = milligram; kg = kilogram; (G) = gavage; d = day; 1x = one time; wk = week; incr = increase; wt = weight; tub = tubular; degen = degeneration.

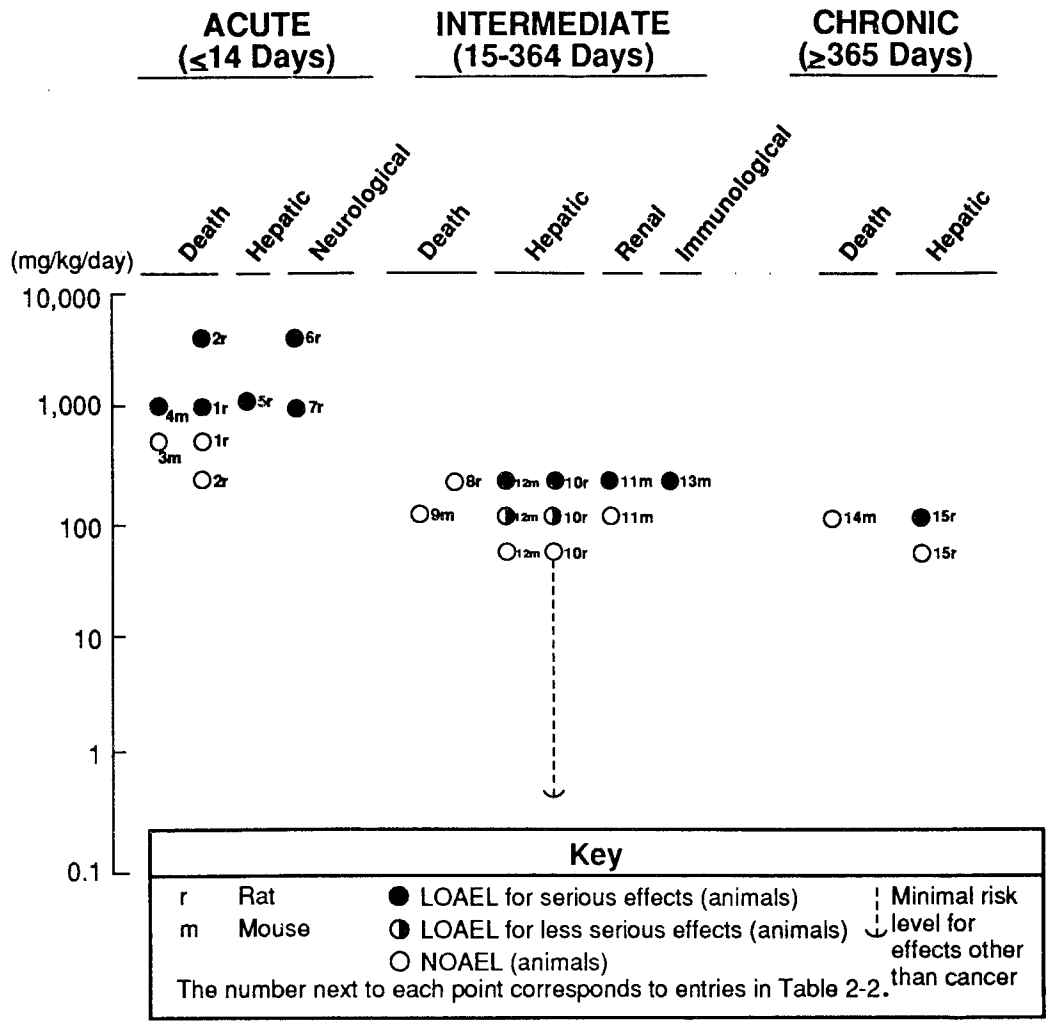


FIGURE 2-2. Levels of Significant Exposure to Chlorobenzene – Oral

The highest NOAEL values and all reliable LOAEL values for renal effects in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.3 Immunological Effects

No studies were located regarding immunological effects in humans following oral exposure to chlorobenzene.

Histological studies in mice and rats suggest that chlorobenzene has immunotoxic properties. Mice exposed to chlorobenzene at ≥250 mg/kg/day by gavage for 13 weeks showed thymic necrosis and lymphoid or myeloid depletion of bone marrow, spleen, or thymus (NTP 1985). While histopathologic evidence suggests that chlorobenzene is immunotoxic, a NOAEL cannot be established in this study since immune function tests were not conducted. A LOAEL of 250 mg/kg/day was determined (NTP 1985). This value has been presented in Table 2-2. Since there are no human data on immunotoxic effects and animal data are sparse, firm conclusions can not be made concerning the potential for chlorobenzene to affect the immune system in humans following oral exposure.

2.2.2.4 Neurological Effects

There is a paucity of data on the effects of chlorobenzene in humans following oral exposure. A two-year-old male swallowed 5 to 10 cc of a stain remover which consisted almost entirely of chlorobenzene. He became unconscious, did not respond to skin stimuli, showed muscle spasms, and became cyanotic. The odor of chlorobenzene could be detected in his urine and exhaled air; however, the child recovered uneventfully (Reich 1934).

No studies were located regarding neurological effects in animals following oral exposure. In the absence of dose-response data in humans and the lack of animal evidence, the potential for chlorobenzene to produce effects on the nervous system cannot be quantitatively determined.

2.2.2.5 Developmental Effects

No studies were located regarding the developmental effects in humans following oral exposure to chlorobenzene.

Limited data in animals suggest that chlorobenzene is not teratogenic. Rats were administered chlorobenzene (100 or 300 mg/kg) in corn oil by gavage from days 6-15 of gestation (IBT 1977). Fetal weight, external anomalies, and skeletal and soft tissue abnormalities

did not differ from control animals in any of the measured parameters. Further, data on maternal weight and behavioral effects did not reveal evidence for dose-related effects.

2.2.2.6 Reproductive Effects

No studies were located regarding the reproductive effects in humans or animals following oral exposure to chlorobenzene.

2.2.2.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or *in vivo* studies in animals following oral exposure to chlorobenzene.

2.2.2.8 Cancer

No studies were located regarding carcinogenic effects in humans following oral exposure to chlorobenzene.

In a chronic oral bioassay in rats and mice (NTP 1985), there was no evidence for carcinogenicity in both sexes of mice or female rats administered chlorobenzene in corn oil by gavage at dose levels up to 120 mg/kg/day. Increased tumor frequencies were not seen in female rats or in male or female mice. Male rats showed a significant ($p < 0.05$) increase in the incidence of neoplastic nodules of the liver in the 120 mg/kg/day dose group, but no increases were found at lower dose levels. While progression from nodules to carcinomas is a well characterized phenomenon, existing data are inadequate to characterize the carcinogenic potential of chlorobenzene in humans. On the basis of these data, the EPA has classified chlorobenzene as a class D carcinogen (i.e., inadequate evidence of carcinogenicity in humans and animals) (EPA 1987c).

2.2.3 Dermal Exposure

No studies were located regarding the following effects in humans or animals following dermal exposure to chlorobenzene.

2.2.3.1 Death

2.2.3.2 Systemic Effects

2.2.3.3 Immunological Effects

2.2.3.4 Neurological Effects

2.2.3.5 Developmental Effects

2.2.3.6 Reproductive Effects

2.2.3.7 Genotoxic Effects

2.2.3.8 Cancer

2.3 TOXICOKINETICS

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

Ogata and Shimada (1983) reported that in two workers exposed to 0.84 and 0.5 ppm of chlorobenzene, the amount absorbed was 38% and 45%, respectively of the administered dose. It should be noted that the percent recovery reported in this study did not take into consideration elimination that occurred during the night nor of expired chlorobenzene. Sullivan et al. (1983) reported that rats readily absorbed ^{14}C-labeled chlorobenzene vapor at concentrations up to 700 ppm.

2.3.1.2 Oral Exposure

Chlorobenzene is absorbed from the gastrointestinal tract. In a study with a single human volunteer, Ogata and Shimada (1983) reported that at least 31% of administered chlorobenzene was absorbed. In the same study, rats administered chlorobenzene absorbed at least 18% of the administered dose. Similar results were reported by Lindsay-Smith et al. (1972), who observed that in rabbits administered ^{14}C-labeled chlorobenzene, at least 22% of the administered chlorobenzene was absorbed.

2.3.1.3 Dermal Exposure

No studies were located regarding dermal exposure to chlorobenzene in humans or animals.

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

No studies were located regarding distribution after inhalation exposure of chlorobenzene in humans.

Sullivan et al. (1983) reported the distribution of ^{14}C-labeled chlorobenzene vapor in rat tissues following single or multiple 8-hour exposures. Some rats were maintained for 48 hours for urine collection. Others were sacrificed immediately or 16 hours after exposure for analysis of tissue radioactivity. The radioactivity in all tissues,

except for fat, increased in proportion to the increase in exposure concentration. The amount of the radiolabel in adipose tissue increased 8 to 10 times when the concentration was increased from 100 to 400 ppm and 3 to 5 times from 400 to 700 ppm. Tissue levels of radioactivity following a single exposure were highest in epididymal and perirenal fat (16.4 and 15.3 micromoles per gram, respectively) after the 700 ppm exposure. These values were not exceeded in animals following multiple exposures. However, multi-exposed rats exhibited higher tissue burdens, 48 hours after the last exposure, than rats exposed only once. The preferential distribution of chlorobenzene to the adipose tissue reflects the lipophilic nature (log octanol/water partition coefficient: 2.84 (Verschueren 1983)) of this compound. The longevity of radioactivity in fat tissue was not determined.

2.3.2.2 Oral Exposure

No studies were located regarding the distribution of chlorobenzene after oral exposure in humans or animals.

2.3.2.3 Dermal Exposure

No studies were located regarding the distribution of chlorobenzene after dermal exposure in humans or animals.

2.3.3 Metabolism

The proposed metabolic pathway (adapted from Ogata and Shimada 1983) of chlorobenzene is shown in Figure 2-3. The main metabolites of chlorobenzene are p-chlorophenylmercapturic acid and 4-chlorocatechol.

The *in vitro* metabolites of chlorobenzene are o-chlorophenol, m-chlorophenol, and p-chlorophenol; the proportions differ according to the source of the mono-oxygenase system and its state of purity (Selander et al. 1975). The o- and p-chlorophenols result from isomerization of the intermediate 3- and 4-chlorobenzene oxides, respectively. The formation of m-chlorophenol appears to occur via a direct oxidative pathway (Oesch et al. 1973). *In vitro* conjugation of the arene oxide with glutathione or hydration is not a significant pathway (Selander et al. 1975).

Ogata and Shimada (1983) examined the urinary metabolites of chlorobenzene in human subjects. An oral dose of 0.3 mmol/kg chlorobenzene was given to a 57-year-old male subject. Metabolites were also assayed in 2 workers exposed via inhalation of either 0.84 or 0.5 ppm of chlorobenzene. They reported the occurrence of 4-chlorocatechol and p-chlorophenylmercapturic acid in the urine of humans who received chlorobenzene orally or by inhalation.

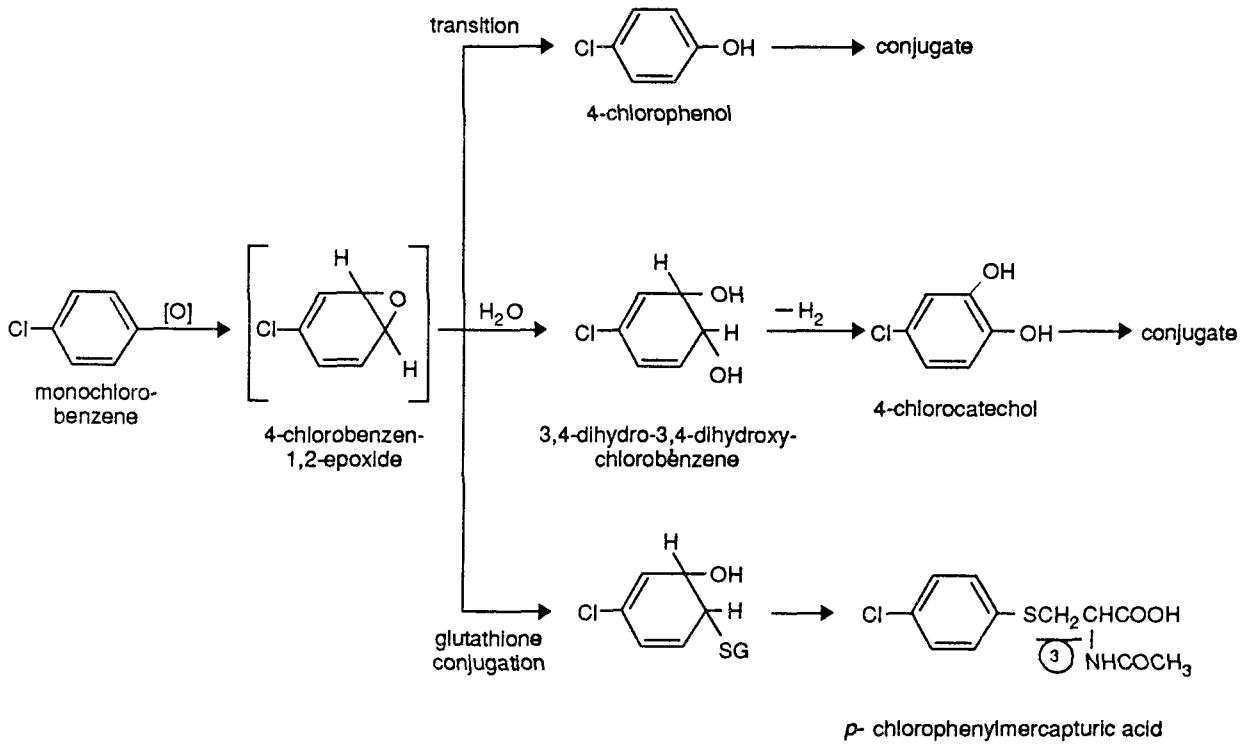


FIGURE 2-3. Metabolic Scheme for Chlorobenzene

Source: Adapted from Ogata and Shimada 1983.

Ogata and Shimada (1983) also examined the urinary metabolites of chlorobenzene in rats, mice, and rabbits. Rats were given oral doses of 0.3 mmol/kg, while all three species received intraperitoneal injections of 0.5, 1.0, or 2.0 mmol/kg. Urinary p-chlorophenylmercapturic acid, and 4-chlorocatechol, after hydrolysis of its conjugate, were reported.

Lindsay-Smith et al. (1972) reported that the major metabolites of chlorobenzene in the rabbit are p-chlorophenylmercapturic acid and conjugates of 4-chlorocatechol. Other urinary metabolites included quinol, 3-chlorocatechol, and o- and m-chlorophenylmercapturic acids. Oesch et al. (1973) studied the metabolism of chlorobenzene in rats administered chlorobenzene by intraperitoneal injection. Thirty-three percent of the administered dose was excreted in the urine, with p-chlorophenol as the major metabolite. Other metabolites included 4-chlorocatechol, o-chlorophenol, and m-chlorophenol.

2.3.4 Excretion

2.3.4.1 Inhalation Exposure

Rats were exposed to ^{14}C-chlorobenzene vapor at concentrations of 100, 400, and 700 ppm for 8 hours (Sullivan et al. 1983). The plasma concentration-time profile for chlorobenzene on cessation of exposure, as estimated by respiratory elimination of radioactivity, indicated a two compartment elimination. Increase in exposure by a factor of seven (100-700 ppm) increased the total uptake of radioactivity by a factor of about 13. This increase in body burden was associated with a decrease in total body clearance, as indicated by an approximate four fold increase in the half-life of the central compartment. The proportion of the dose excreted via the lungs (which may be presumed to be largely, if not entirely, unchanged chlorobenzene) increased nonlinearly and the proportion eliminated by hepatic metabolism decreased. Increase in the dose of chlorobenzene was associated with a decrease in the proportion cleared as the mercapturic acid derivative. Of interest, the half-life of chlorobenzene was shorter at the 700 ppm exposure level when the animals were subjected to repeated treatment daily for 5 days, as compared with that of the single 700 ppm exposure animals, raising the possibility of induction of metabolic clearance. In agreement with this possibility, the proportion cleared by metabolism in the multi-exposed animals was increased, and the proportion excreted unchanged via the lung was decreased, as compared with the 700 ppm-single exposure animals.

Ogata and Shimada (1983) reported that in two workers exposed to 0.84 and 0.5 ppm of chlorobenzene, the excretion of p-chlorophenylmercapturic acid was markedly lower than that of 4-chlorocatechol. However, the ratio of mercapturic-acid to

4-chlorocatechol in the urine of human subjects receiving chlorobenzene orally was similar to that of workers inhaling chlorobenzene.

2.3.4.2 Oral Exposure

Ogata and Shimada (1983) also assayed the urinary metabolites of chlorobenzene of a 57-year-old male volunteer given an oral dose of 0.3 mmol/kg of chlorobenzene. Two urinary metabolites, p-chlorophenylmercapturic acid and 4-chlorocatechol, were detected. As in the case of inhalation exposure, the excretion of p-chlorophenylmercapturic acid was reported to be markedly lower than that of 4-chlorocatechol. However, the ratio of mercapturic-acid to 4-chlorocatechol in the urine of human subjects receiving oral chlorobenzene was similar to that of workers inhaling chlorobenzene.

Lindsay-Smith et al. (1972) reported that rabbits administered ^{14}C-labeled chlorobenzene excreted 22% of the radiolabel in the urine. The authors concluded that the remaining radiolabel was excreted in the expired air. Ogata and Shimada (1983) reported that in rats the primary urinary metabolite was p-chlorophenylmercapturic acid and that 4-chloroatechol was a minor metabolite.

2.3.4.3 Dermal Exposure

No studies were located concerning excretion of chlorobenzene in animals or man after dermal exposure.

2.4 RELEVANCE TO PUBLIC HEALTH

Inhalation studies in humans and animals and oral studies in animals demonstrate that chlorobenzene can affect the central nervous system, liver, and kidneys. Chlorobenzene did not affect the developing fetus, was not genotoxic, and did not affect reproduction. Data has not provided clear evidence that chlorobenzene causes cancer in animals. Existing data are considered inadequate to derive human minimal risk levels for acute and chronic exposures.

Death. No case studies of human fatalities have been reported following exposure to chlorobenzene by inhalation, ingestion, or dermal contact. Death has been reported in animals at high doses for brief periods of exposure. Rabbits died within 2 weeks after removal from exposure at approximately 537 ppm (Rozenbaum et al. 1947). The cause of death has been attributed to central nervous system depression resulting in respiratory failure. Animal data suggest that lethality may not be a concern for humans unless the exposure level is very high.

2. HEALTH EFFECTS

Systemic Effects. No studies were located regarding effects on the respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, or dermal/ocular systems in humans or animals by any route of exposure to chlorobenzene.

Hepatic Effects. No studies were located demonstrating that chlorobenzene causes hepatic toxicity in humans by any route of exposure. Acute and intermediate exposures in animals demonstrated that chlorobenzene causes changes in liver weights and enzyme levels, degeneration, necrosis, and alterations in microsomal enzymes. These effects were first evident during acute exposure (5 days) at 1,140 mg/kg/day by gavage (Rimington and Ziegler 1963) and intermediate exposure (5 days/wk for 24 weeks) at 75 ppm via inhalation (Dilley 1977). Similar effects were also observed following ingestion of $\geq$250 mg chlorobenzene/kg/day for 91 days. The precise mechanism for liver damage is not known; however, direct binding of chlorobenzene metabolites to cellular protein may be involved (Reid et al. 1973; Reid and Krishna 1973). There were differential sensitivities in animal species tested which may be due to differences in metabolism. Based on animal studies, liver toxicity may be an area of concern in humans.

Renal Effects. No studies were located demonstrating that chlorobenzene causes renal effects in humans by any route of exposure. Intermediate studies in animals showed effects on the kidney at doses comparable to those causing liver effects. Typical signs included tubular degeneration and necrosis as well as changes in organ weight. Changes in organ weights with accompanying histopathology occurred at $\geq$250 mg/kg/day (90 days) (Kluwe et al. 1985). The precise mechanism of kidney damage is not clear. However, necrosis was associated with covalent binding of substantial amounts of radiolabeled chlorobenzene to kidney protein in intraperitoneal studies (Reid 1973). This study also reported that autoradiograms revealed that most of the covalently bound material was localized within necrotic tubular cells (Reid 1973). Based on animal studies, renal toxicity may be an area of concern in humans.

Immunological Effects. Histopathologic evaluations in animals suggest that chlorobenzene may be immunotoxic; however, direct tests of immune function have not been performed. In the absence of functional assessment, the potential for chlorobenzene to affect the immune system in humans can not be determined.

Neurological effects. Case reports of humans demonstrated that chlorobenzene caused disturbances of the central nervous system, but there were no reports of changes in the structure of the brain and other parts of the nervous system. Effects were observed in humans who inhaled vapors of chlorobenzene in the workplace for up to 2 years (Rozenbaum et al. 1947). Effects included headaches, dizziness, and

sleepiness. Unconsciousness, lack of response to skin stimuli, and muscle spasms were noted following accidental ingestion. While there is qualitative evidence for central nervous system effects in humans, a quantitative assessment can not be made since exposure levels were not reported. Because work practices have changed significantly since these studies, it is reasonable to assume that exposure levels in this study were higher than current permissible federal exposure levels. Acute studies in animals confirm that chlorobenzene is potentially neurotoxic. These effects appear to be the result of narcotic effects of chlorobenzene on the central nervous system. Acute inhalation exposure produced narcosis preceded by muscle spasms in rabbits at 1,090 ppm (Rozenbaum et al. 1947).

Developmental Effects. No studies were found regarding the developmental toxicity of chlorobenzene in humans. In inhalation and oral exposure studies, the animals did not demonstrate significant developmental toxicity when compared with untreated controls. Negative responses in two animal species suggest that developmental toxicity may not be an area of concern for chlorobenzene.

Reproductive Effects. No studies were found regarding the reproductive toxicity of chlorobenzene in humans. In a two-generation inhalation study, chlorobenzene did not adversely affect various reproductive parameters in rats (Nair et al. 1987). While results of this study suggest reproductive toxicity may not be an area of concern to humans, other considerations are warranted before firm conclusions can be made regarding risk to humans. The slight increase in the occurrence of degeneration of the germinal epithelium of the testes provides some evidence for further consideration. Also, the study did not provide histopathological data on other organs related to reproductive functions (i.e., epididymis, vas deferens, accessory sex glands, and pituitary). While the authors reported no treatment-related impairment of fertility, it should be noted that fertility assessments in test animals are limited by their insensitivity as measures of reproductive injury in humans.

Genotoxic Effects. No studies were located regarding the genotoxic effects of chlorobenzene in humans. No in vivo animal assays were found, except the micronuclear test in mice which was moderately positive (Mohtashamipur et al. 1987) (Table 2-3). Furthermore, in vitro tests employing bacterial and yeast assay systems with and without metabolic activation were negative (Haworth et al. 1983; NTP 1985; Prasad 1970). Chlorobenzene induced transformation in adult rat liver epithelial cells but was not genotoxic to hepatocytes (Shimada et al. 1983). Since transformations may occur through nongenotoxic mechanisms, results do not necessarily indicate that chlorobenzene is potentially genotoxic. Results of in vitro assays for chlorobenzene are presented

TABLE 2-3. Genotoxicity of Chlorobenzene In Vivo

End Point	Species (Test System)	Exposure Route	Results	Reference
Mammalian systems:				
Chromosomal	Mouse (micronuclear)	IP	+	Mohtashumipur et al. 1987

IP = intraperitoneal; + = positive result.

in Table 2-4. Existing data suggest that genotoxicity may not be an area of concern for chlorobenzene exposure in humans.

Cancer. No studies were found regarding the carcinogenicity of chlorobenzene in humans. In a chronic bioassay in animals, chlorobenzene (up to 120 mg/kg/day) did not produce increased tumor incidences in mice of both sexes or in female rats (NTP 1985). It was noted, however, that male rats showed a statistically significant increase in neoplastic nodules at the highest dose level tested. While there is strong evidence for neoplastic nodules, existing data are inadequate to characterize the potential for chlorobenzene to cause cancer in humans and animals.

2.5 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time biologic samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to chlorobenzene are discussed in Section 2.5.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial

TABLE 2-4. Genotoxicity of Chlorobenzene *In Vitro*

End Point	Species (Test System)	Results: With Activation	Results: Without Activation	Reference
Prokaryotic organisms:				
Gene mutation	*Salmonella typhimurium*	-	-	NTP 1985
Gene mutation	*S. typhimurium*	-	-	Haworth et al. 1983
Eukaryotic organisms:				
Fungi:				
Gene mutation	*Aspergillus nidulans*	-	No data	Prasad 1970
Mammalian cells:				
Genetic endpoint unknown	Rat (cellular transformation)	+	No data	Shimada et al. 1983
DNA Repair	Rat (UDS)	-	No data	Shimada et al. 1983

- = negative result; + = positive result; UDS = unscheduled DNA synthesis.

cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers a often not substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by chlorobenzene are discussed in Section 2.5.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, biologically effective dose, or target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.7, "POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE."

2.5.1 Biomarkers Used to Identify and/or Quantify Exposure to Chlorobenzene

Levels of chlorobenzene and its metabolites have been measured in blood, urine, and exhaled air; however, no studies were located linking any level of chlorobenzene in humans with a biological effect. Levels ranging from 0.05 to 17 mg/L were detected in the blood and 25 to 120 μg/L in the urine of residents living near a former toxic chemical dump, while trace amounts were found in exhaled air (Barkley et al. 1980).

2.5.2 Biomarkers Used to Characterize Effects Caused by Chlorobenzene

Neurological damage is a characteristic biomarker of effect in humans exposed to chlorobenzene. Additional information on health effects associated with exposure to chlorobenzene can be found in Section 2.2. Various clinical signs and symptoms of people exposed to chlorobenzene which may be monitored include headaches, dizziness, muscle spasms, and cyanosis (from respiratory depression). No data we found on biochemical changes which may exist.

Studies in animals suggest that chlorobenzene may also cause injury to the liver. In rats, alkaline phosphatase, SGOT, and delta-amino levulinic acid levels were increased as were liver protoporphyrin and uroporphyrin. Data suggest that the kidneys may be affected following exposure to chlorobenzene as polyuria was noted in rats at high dose levels. Since other chemicals may produce similar effects, these are not specific indicators of chlorobenzene exposure.

2.6 INTERACTIONS WITH OTHER CHEMICALS

In an attempt to identify the proposed epoxide intermediate of chlorobenzene, Oesch (1973) co-administered the epoxide hydrase

inhibitor cyclohexane oxide together with chlorobenzene to rats. Instead of increasing the toxicity of chlorobenzene as expected, through the inhibition of epoxide hydrase, cyclohexane oxide actually decreased the metabolism of chlorobenzene and its necrotic toxicity on the liver, suggesting that the metabolism of chlorobenzene is partially responsible for its liver toxicity.

2.7 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

No studies were located regarding human populations that are unusually susceptible to chlorobenzene. By analogy to other lipophilic chlorinated benzenes such as hexachlorobenzene, which is found in human milk (Weisenberg et al. 1985), nursing infants may be susceptible to chlorobenzene toxicity.

2.8 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of chlorobenzene is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of chlorobenzene.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that, if met would reduce or eliminate the uncertainties of human health assessment. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

2.8.1 Existing Information on Health Effects of Chlorobenzene

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to chlorobenzene are summarized in Figure 2-4. The purpose of this figure is to illustrate the existing information concerning the health effects of chlorobenzene. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not imply anything about the quality of the study or studies. Gaps in this figure should not be interpreted as "data needs" information.

As summarized in Figure 2-4, there is a paucity of data on health effects of chlorobenzene in humans. Existing data relate to inhalation and oral exposures. No data were found on dermal exposures.

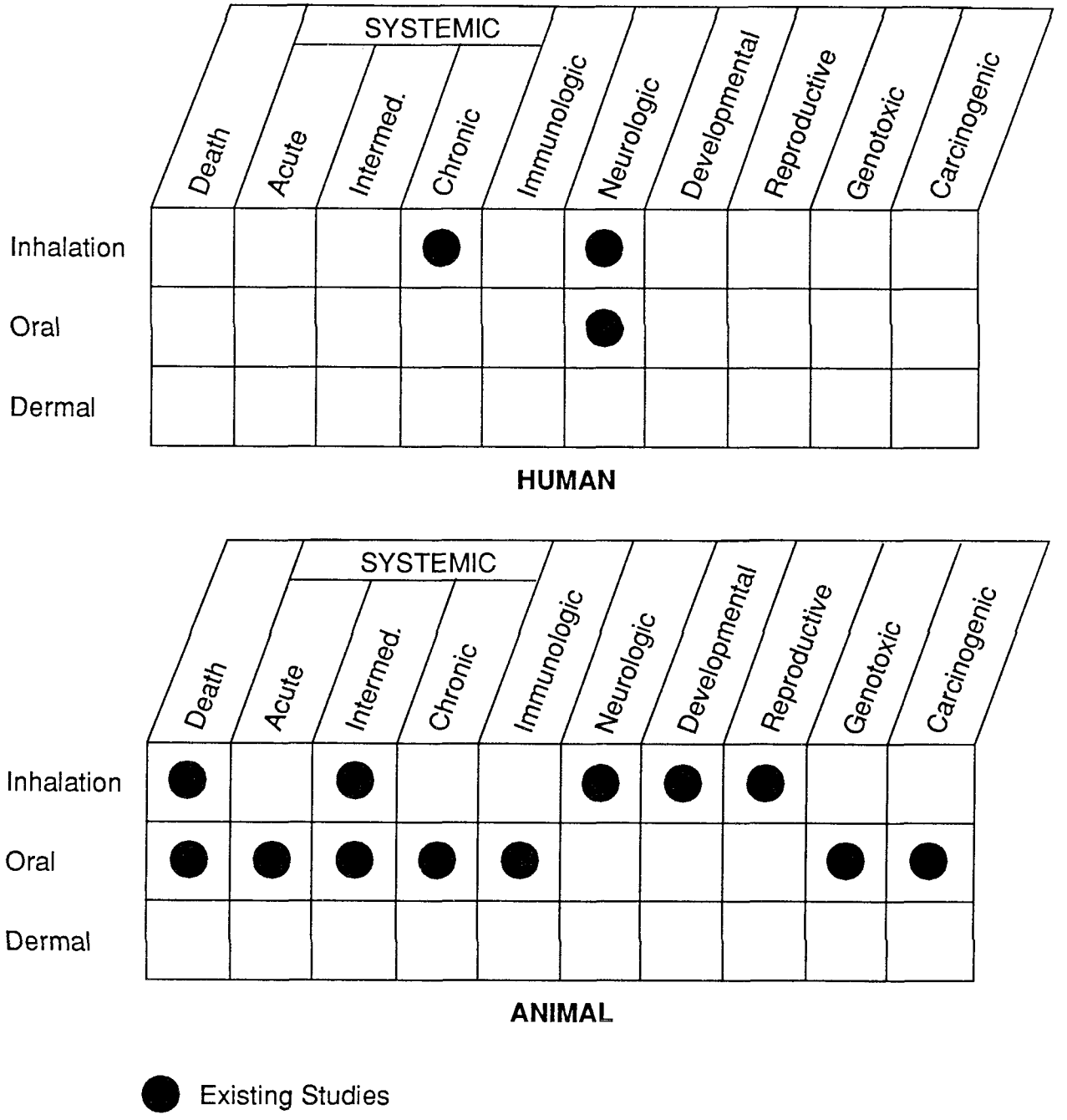


FIGURE 2-4. Existing Information on Health Effects of Chlorobenzene

The toxicity of chlorobenzene has been studied in animals by oral and inhalation exposures, but there are no data on dermal exposures. Oral studies have focused on systemic toxicity (liver and kidney) and genotoxic and carcinogenic effects. There are inhalation studies evaluating neurologic, developmental, and reproductive effects.

2.8.2 Identification of Data Needs

Acute-Duration Exposure. No information is available on the effects of acute-duration exposure of humans to chlorobenzene by any route of exposure. Animal studies indicate that acute inhalation and oral exposures can result in death. No other treatment-related effects were reported. There are no data on effects of chlorobenzene following dermal exposure in animals. Since data on effects in humans are not available and animal data are limited to lethality, data are not sufficient to derive an acute MEL. Further studies would be useful to identify target tissues and threshold levels for effects that may exist.

Intermediate-Duration Exposure. No studies are available in humans on the effects of intermediate-duration exposure to chlorobenzene by any route. Inhalation and oral studies in animals indicate that the nervous system, liver, and kidneys are principal target tissues following exposure to chlorobenzene. An intermediate oral MRL was derived based on liver effects in rats. There are no data on effects following dermal exposure in animals. Because there is potential for exposure to chlorobenzene through skin contact, additional studies by dermal exposure would add to the database on chlorobenzene toxicity.

Chronic-Duration Exposure and Cancer. Limited studies are available on the effects in humans chronically exposed to chlorobenzene via inhalation and suggest that nervous system is a target tissue. Specific exposure data were not provided. No information is available on effects of chlorobenzene in humans following chronic oral or dermal exposure. Inhalation and oral studies in animals identified the same target tissues as for intermediate-duration exposure. One study in rats demonstrated that the immune system can also be adversely affected via oral exposure. Inhalation studies in humans and inhalation and oral studies in animals are sufficient to identify main target tissues. A chronic MRL was not derived since human exposure data were lacking and the one animal study did not evaluate a sufficient number of end points and test animals. Further studies via the dermal route would provide additional toxicity data for an assessment of potential risk to humans.

No studies were found in humans regarding the carcinogenic effect of chlorobenzene via inhalation. Since this is the primary route of environmental exposure, additional studies would be useful to assess potential risk to people who may be exposed to low levels of

chlorobenzene in air near hazardous waste sites. There was no evidence for carcinogenicity in both sexes of mice or female rats following oral exposure to chlorobenzene. Since the animals were tested at the maximum tolerated dose and a no-effect level for tumors in rats and mice has been determined, additional oral studies are not warranted at this time.

Genotoxicity. No studies were found on the genotoxic effects of chlorobenzene in humans by any route of exposure. Results of animal assays were mixed. Chlorobenzene induced statistically significant increases in polychromatic erythrocytes containing micronuclei in mice following intraperitoneal injections. Results of cellular transformation assays of rat liver epithelial cells were positive, but chlorobenzene did not induce unscheduled DNA synthesis in primary rat hepatocytes. Studies evaluating the mutagenic potential of chlorobenzene have been negative. Since existing data do not suggest a significant genotoxic risk associated with exposure to chlorobenzene, additional studies are not warranted at this time.

Reproductive Toxicity. No studies were found on the reproductive toxicity of chlorobenzene by any route in humans. Chlorobenzene did not affect various reproductive parameters in a two-generation inhalation study in rats. Additional animal studies employing another species would provide further information for assessing potential effects on the reproductive functions of chlorobenzene. These studies should provide histological evaluations of organs related to reproduction function (i.e., epididymis, vas deferens, accessory sex glands, and pituitary) since these organs have not been evaluated. Slight increases in the incidence of degeneration of testicular epithelium are also noteworthy for further consideration.

Developmental Toxicity. No studies have been conducted to evaluate the developmental toxicity of chlorobenzene in humans. Chlorobenzene did not affect the developing fetus following inhalation and oral exposures by rats and rabbits. While there is a potential for exposure via the dermal route, the absence of significant effects by the primary exposure route (inhalation) suggests that additional studies may not be needed at this time.

Immunotoxicity. There are no data available on the immunotoxicity of chlorobenzene in humans by any route of exposure. Histological examination of organs and tissues of the immunological system in mice and rats provide some evidence that chlorobenzene is potentially immunotoxic. Immune function tests would provide a better assessment of potential immunotoxic effects in humans.

Neurotoxicity. Limited data in humans indicate that exposure to chlorobenzene via inhalation and oral exposures can result in effects on

the nervous system. Clinical signs and symptoms were observed, but histological lesions were not reported. Results of inhalation studies in animals confirm clinical aberrations, but no data were found in animals following oral exposure. Further studies employing other animal species and various dose levels would be useful to determine if similar effects exist following oral and dermal exposures. Although the inhalation of contaminated air is the most probable route of exposure to chlorobenzene, there is also potential for exposure through skin contact or by consumption of contaminated water. Animal studies in which chlorobenzene is administered orally or dermally would allow determination of neurotoxicity by these routes.

Epidemiological and Human Dosimetry Studies. No epidemiological studies have been conducted to evaluate the adverse health effects of chlorobenzene. Existing studies are limited to case reports of occupational exposures and identified the nervous system as a target tissue following chronic inhalation of chlorobenzene. Reliable exposure data were not reported. Additional studies which provide quantitative exposure data would be useful in evaluating potential risk to humans and providing a better understanding of levels which lead to effects that may exist.

Biomarkers of Exposure and Effect. Parent chlorobenzene and metabolites can be detected in biological tissues and fluids. However, existing methods may not be useful for evaluating the general population as opposed to industrial situations where preexposure levels are established prior to known chlorobenzene exposure. The overall reliability of these biomarkers are further reduced since data are not available on the half-life of chlorobenzene in various biological media.

Central nervous system injury is a common effect associated with exposure to vapors of chlorobenzene in humans. Studies in animals suggest that chlorobenzene can also result in damage to the liver and kidneys. Since similar effects occur with exposure to other chemicals, additional studies are needed to identify more specific biomarkers by which to monitor populations living near hazardous waste sites.

Absorption, Distribution, Metabolism, and Excretion. The toxicokinetics of chlorobenzene have not been evaluated to any great extent in humans. Limited studies suggest that chlorobenzene can be absorbed following inhalation and oral exposures, but no data were found on absorption following dermal exposure. Based on absorption characteristics of benzene and the high lipid solubility of chlorobenzene, absorption may be significant depending on conditions. Additional studies are needed to determine absorption rates following exposure by all routes.

Data are also sparse on the distribution of chlorobenzene. No information is available regarding distribution of chlorobenzene in humans by inhalation, oral, or dermal exposure. There are limited animal data which suggest preferential distribution to adipose tissue in rats via inhalation. The kidneys and liver also showed significant amounts of chlorobenzene and rats that received multiple doses exhibited higher tissue burdens than rats exposed only once.

The metabolic transformation of chlorobenzene has been evaluated in humans and animals. Although ultimate products of metabolic oxidation are known, the oxidative pathway and possible intermediates have not been established. Principal metabolites have been determined but quantities and ratios differ among species. Additional studies would be useful to determine if these differences affect the toxicity of chlorobenzene.

There are limited data on the excretion of chlorobenzene. In humans exposed via the inhalation and oral routes, chlorobenzene and its metabolites were detected in urine and there were differences in excretion patterns via the two routes. Chlorobenzene and its metabolites were also detected in exhaled air of rats following inhalation and in exhaled air and urine in rabbits after oral exposure. The urinary metabolite profile appeared to be dose dependent and there were changes in excretion patterns due to multiple versus single exposures. No data on excretion following dermal exposure are available. Additional studies would be useful in determining the significance of these differences with regard to risk associated with different routes of exposure.

Comparative Toxicokinetics. Existing studies regarding toxicokinetics of chlorobenzene in humans are limited, but data do provide some understanding of the absorption, metabolism, and excretion following inhalation and oral exposures. Since studies on distribution of chlorobenzene are lacking, quantitative data correlating human exposure and tissue accumulation would be useful. In animals, quantitative data on absorption, distribution, metabolism, and excretion are very limited in extent and quality. Additional studies using a variety of species and including physiological based pharmacokinetic modeling would be useful in determining the most suitable animal model for assessing human risk.

2.8.3 On-going Studies

Chlorobenzene is one of 47 chemicals to be tested by NTP for heritable genetic effects in Drosophila and for mutagenesis in the mouse lymphoma cell mutagenesis assay.

3. CHEMICAL AND PHYSICAL INFORMATION

3.1 CHEMICAL IDENTITY

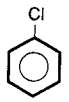
Table 3-1 lists common synonyms, trade names and other pertinent identification information for chlorobenzene.

3.2 PHYSICAL AND CHEMICAL PROPERTIES

Table 3-2 lists important physical and chemical properties of chlorobenzene.

3. CHEMICAL AND PHYSICAL INFORMATION

TABLE 3-1. Chemical Identity of Chlorobenzene

Characteristic	Value	Reference
Chemical name	Chlorobenzene	NLM 1988 NLM 1988
Synonyms	Monochlorobenzene; benzene chloride; phenylchloride; MCB; chlorobenzol	NLM 1988
Trade name	Caswell no. 183A	NLM 1988
Chemical formula	C_6H_5Cl	NLM 1988
Chemical structure		
Identification numbers:		
CAS Registry	108-90-7	NLM 1988
NIOSH RTECS	CZ0175000	HSDB 1988
EPA Hazardous Waste	U037,F002	HSDB 1988
OHM/TADS	No data	
DOT/UN/NA/IMCO Shipping	UN 1134 IMCO 3.3	NLM 1988 HSDB 1988
HSDB	55	NLM 1988
NCI	C54886	NLM 1988

CAS = Chemical Abstracts Service; NIOSH = National Institute for Occupational Safety and Health; RTECS = Registry of Toxic Effects of Chemical Substances; EPA = Environmental Protection Agency; OHM/TADS = Oil and Hazardous Materials/Technical Assistance Data System; DOT/UN/NA/IMCO = Department of Transportation/United Nations/North America/International Maritime Dangerous Goods Code; HSDB = Hazardous Substances Data Bank; NCI = National Cancer Institute.

TABLE 3-2. Physical and Chemical Properties of Chlorobenzene

Property	Value	Reference
Molecular weight	112.56	Weast 1985
Color	Colorless	Verschueren 1983
Physical state	Liquid	Verschueren 1983
Melting point	-45.6°C	Weast 1985
Boiling point	132°C	Weast 1985
Density at 20°C	1.1058	Weast 1985
Odor	Aromatic, almond-like	Sax and Lewis 1987
Odor threshold:		
Water	0.050 mg/L	Verschueren 1983
Air	1-8 mg/m^3	Verschueren 1983
Solubility:		
Water at 20°C	500 mg/L	Verschueren 1983
Organic solvents	Soluble in alcohol, ether, benzene	Weast 1985
Partition coefficients:		
Log octanol/water	2.84	Verschueren 1983
Log K_{oc}	2.52	Mabey et al. 1982
Vapor pressure at 20°C	8.8 mmHg	Verschueren 1983
Henry's law constant	3.58×10^{-3} atm-m^3/mol	Mabey et al. 1982
Autoignition temperature	637°C	Sax and Lewis 1987
Flashpoint	29.4°C	Sax and Lewis 1987
Flammability limits	1.8%-9.6%	Sax and Lewis 1987
Conversion factors	1 ppm = 4.7 mg/m^3 1 mg/m^3 = 0.22 ppm	Verschueren 1983

4. PRODUCTION, IMPORT, USE, AND DISPOSAL

4.1 PRODUCTION

Production of chlorobenzene in the United States has declined by nearly 60%, from the peak production volume of 274,000 kkg in 1960 to 112,000 kkg in 1987. This decline is attributed primarily to the replacement of chlorobenzene by cumene in phenol production and the cessation of DDT production in the United States. In addition, pesticide production using chlorobenzene as an intermediate has declined and no major new uses have been found for chlorobenzene in recent years. Therefore, the decline in chlorobenzene production is expected to continue (EPA 1980c, 1985; Hughes et al. 1983; USITC 1988).

Chlorobenzene is produced by three United States chemical companies: Monsanto Chemical Company, Sauget, Illinois; PPG Industries, Inc., Natrium, West Virginia; and Standard Chlorine Chemical Co., Inc., Delaware City, Delaware. Production capacity for chlorobenzene at these plants has remained constant since 1985 although it appears that actual production has declined slightly during that period (Hughes et al. 1983; SRI 1985, 1986, 1987, 1988; USITC 1988).

Chlorobenzene is produced commercially by the chlorination of benzene in the presence of a catalyst (e.g., ferric chloride, aluminum chloride, or stannic chloride). This process yields a mixture of chlorobenzene, dichlorobenzenes, and higher analogs which are distiller and crystallized to obtain pure products (EPA 1985a; Hughes et al. 1983).

4.2 IMPORT

Import and export data for chlorobenzene are not readily available. Estimates indicate that for the last ten years, both imports and exports have been negligible (Hughes et al. 1983).

4.3 USE

The current primary uses of chlorobenzene are as a solvent for pesticide formulations, diisocyanate manufacture, degreasing automobile parts, and for the production of nitrochlorobenzene. Solvent uses accounted for about 37% of chlorobenzene consumption in the United States in 1981, nitrochlorobenzene production for 33%, and diphenyl oxide and phenylphenol production for 16% of consumption. Chlorobenzene

is also used in silicone resin production and as an intermediate in the synthesis of other halogenated organics. The past major use of chlorobenzene was as an intermediate in phenol and DDT production (Hughes et al. 1983).

4.4 DISPOSAL

Because chlorobenzene is listed as a hazardous substance, disposal of waste chlorobenzene is controlled by a number of federal regulations (see Chapter 7). Spent solvent wastes, which may include chlorobenzene, are prohibited from land disposal, except under specific conditions. Land disposal restrictions (treatment standards) are proposed for other wastes containing chlorobenzene. Wastes containing chlorobenzene may be disposed of by liquid injection, rotary kiln, or fluidized bed incineration (EPA 1988a, 1989b; HSDB 1988). Since chlorobenzene is a volatile compound and is used extensively as a solvent, large quantities are released to the air. Some estimates indicate that 30 to 50% of the annual production of chlorobenzene is released to the atmosphere, while less than 0.1% is found in wastewater and less than 1% is disposed of on land (EPA 1985a).

5. POTENTIAL FOR HUMAN EXPOSURE

5.1 OVERVIEW

Chlorobenzene is used as a solvent and as an intermediate in industry. A portion of that is lost to the environment in water and air discharges. Chlorobenzene adsorbs moderately to soil and is biodegraded comparatively rapidly. With a moderate index of bioaccumulation, chlorobenzene was found in almost every individual tested for it in the United States. The EPA has identified 1,177 NPL sites. Chlorobenzene has been found at 97 of the sites evaluated for the presence of this chemical. As more sites are evaluated by the EPA, the number may change. The frequency of these sites within the United States can be seen in Figure 5-l.

5.2 RELEASES TO THE ENVIRONMENT

5.2.1 Air

The production of chlorobenzene by seven major producers was reported to be 112,000 kkg in 1987. Estimates of environmental releases vary widely. The EPA (1982d) estimated the release of chlorobenzene to be about 200 tons, or 0.2% of production, while Dow Chemical Company estimated that about 50,000 tons, or 30% to 50% of their annual production was released to the air (EPA 1980a).

5.2.2 Water

The principal source of chlorobenzene in water is release from chemical manufacturing facilities. Dow Chemical Company estimated that 0.1% of its annual production enters waters (EPA 1980a). Perry et al. (1979) found chlorobenzene in 6/63 industrial effluent in concentrations up to 100 μg/L. Based on 1,338 samples collected from about 1980 to 1983, the medium concentration of chlorobenzene in waste effluent was < 3 ppb and was detected in 54 samples. The total amount released to the environment was not reported (Staples et al. 1985). Chlorobenzene has been detected in both surface and groundwater samples at hazardous waste sites. Data from the Contract Laboratory Program (CLP) Statistical Database indicate that chlorobenzene occurred in surface water at 13 sites at a geometric mean concentration of 17 ppb in positive samples and in groundwater at 28 sites at a geometric mean concentration of 62 ppb in positive samples (CLPSD 1988). It should be noted that the CLP Statistical Database includes data from both NPL and non-NPL sites.

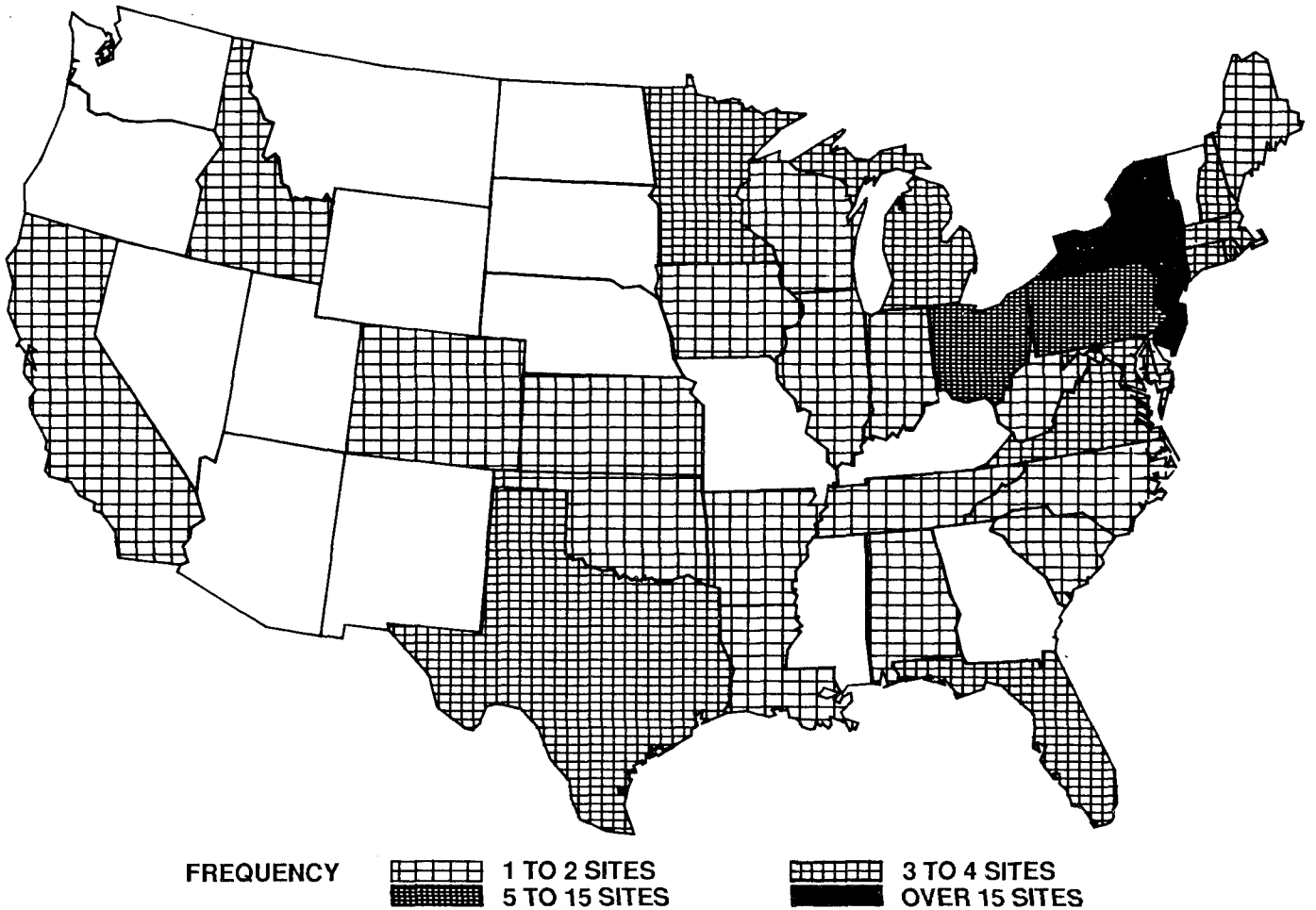


FIGURE 5-1. Frequency of Sites with Chlorobenzene Contamination

5.2.3 Soil

Chlorobenzene was detected at 34 sites at a geometric mean concentration of 37 ppm in positive soil samples (CLPSD 1988). It should be noted that the CLP Statistical Database includes data from both NPL and non-NPL sites.

5.3 ENVIRONMENTAL FATE

5.3.1 Transport and Partitioning

Chlorobenzene is volatile and has only moderate solubility in water (500 mg/L). Chlorobenzene was observed to evaporate ($\geq$ 99%) from an unaerated aqueous solution in 72 hrs (Garrison and Hill 1972). The air, undoubtedly, plays a large role in the environmental transport and degradation of chlorobenzene, although studies addressing this aspect were not found.

5.3.2 Transformation and Degradation

5.3.2.1 Air

Physical constants for chlorobenzene, especially its vapor pressure and water solubility, indicate that the air is an important and perhaps the dominant medium for the transport and transformation of chlorobenzene. As an aromatic molecule with strong UV-absorption, chlorobenzene has a half-life of 20 to 40 hrs under simulated atmospheric conditions (Dilling et al. 1976). This appears to be confirmed by the large difference between chlorobenzene measurements in urban air (3,000 ng/m^3) and in rural air (not detected) in 1982 (Brodzinsky and Singh 1983).

5.3.2.2 Water

Biodegradation in a waste water inoculum was studied by Tabak et al. (1981). Among 57 environmental pollutants tested, chlorobenzene at 5 mg/L was among the more rapidly biodegraded substances with 89% degradation in a week and 100% after adaptation. Biodegradation is therefore a major degradation process in oxygenated waters while evaporation will play an additional role in surface waters.

5.3.2.3 Soil

Biodegradation of chlorobenzene is rapid, leaving no detectable residues after 1 or 2 weeks. Adaptation is also rapid (Tabak et al. 1981).

5.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

5.4.1 Air

Air samples at 56 localities in the United States in 1982 had mean chlorobenzene concentrations of about 3,000 ng/m^3; the highest concentrations in urban and suburban areas, at much lower levels at the sites of production, but was not detectable in rural and remote areas (Brodzinsky and Singh 1983). This suggests a substantial contribution to urban air levels by small industry and consumer products but also a short residence time in the air. A study of New Jersey waste sites found similar air levels of chlorobenzene (2,500 ng/m^3) (Harkov et al. 1985). However, air levels found by another study done for the United States EPA (Pellizzari 1978a) were an order of magnitude lower, with only the air over a waste site approaching the mean urban concentrations reported above. Ambient air outside homes of "Old Love Canal" (Niagara Falls, New York) contained chlorobenzene ranging from not detectable (4 sites) to traces (4 sites) and 120 ng/m^3 (1 site) (Barkley et al. 1980).

5.4.2 Water

Chlorobenzene, along with other chlorinated chemicals, was found in United States' rivers at levels up to and exceeding 10,000 ng/L (Shackelford and Keith 1976; Sheldon and Hites 1978). Private wells near a hazardous waste site contained as much as 41 μg/L (Clark 1982) and tap water at Love Canal contained 10 to 60 ng/L of chlorobenzene (Barkley et al. 1980).

Chlorobenzene contamination of industrial waste waters up to and exceeding 100 μg/L was found in 6/63 samples (Perry et al. 1979) and in 147/31,194 samples with a mean concentration of 667 μg/L (EPA 1985a).

5.4.3 Soil

Staples et al. (1985) reported that the median concentration of chlorobenzene in the United States was estimated to be less than 5 ppb dry sediments. In 347 measurements recorded in the STORET data base, 2% of the samples contained detectable concentrations of chlorobenzene.

5.4.4 Other Media

No studies of chlorobenzene in food or other media are available.

5.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

Chlorobenzene was found in 98/100 human adipose tissue samples from all regions of the United States at levels ranging from 1 to 9 ng/g (Stanley 1986). At Love Canal, Niagara Falls, chlorobenzene could be

detected in the breath of one of nine people evaluated for exposure and in the urine of six of nine persons at 20 to 120 ng/L (Barkley et al. 1980).

Personal sampling at chemical companies (Cohen et al. 1981) indicated that chlorobenzene levels (up to 18 mg/m^3) in work place air did not exceed the current federal level (350 mg/m^3).

5.6 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Occupational settings provide the greatest potential for high exposures to chlorobenzene. Since chlorobenzene is a volatile compound and is used extensively as a solvent, large quantities may be released to the workplace air. Other populations who might be exposed include persons living near industrial facilities where chlorobenzene emissions are not properly controlled.

5.7 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of chlorobenzene is available. Where adequate information is not available, ATSDR, in conjunction with the NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of chlorobenzene.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that, if met would reduce or eliminate the uncertainties of human health assessment. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

5.7.1 Identification of Data Needs

Physical and Chemical Properties. Physical and chemical properties of chlorobenzene have been thoroughly measured.

Production, Use, Release, and Disposal. Data indicate that chlorobenzene production has declined dramatically over the past two decades, but current quantitative data on use (especially solvent uses) and disposal practices would be helpful in evaluating the effect of current industrial practices on environmental levels of chlorobenzene.

5. POTENTIAL FOR HUMAN EXPOSURE

According to the Emergency Planning and Community Right to Know Act of 1986 (EPCRTKA), (§313), (Pub. L. 99-499, Title III, §313), industries are required to submit release information to the EPA. The Toxic Release Inventory (TRI), which contains release information for 1987, became available in May of 1989. This database will be updated yearly and should provide a more reliable estimate of industrial production and emission.

Environmental Fate. Information on biodegradation in soil under aerobic conditions exists, but degradation products were not identified. Anaerobic biodegradation, as might occur in river bottoms and in Superfund sites, has not been studied and would be valuable. Emissions from waste lagoons have been modelled and measured in bench-top experiments and are measured as part of many Superfund Remedial Investigation/Feasibility studies, but those were not located.

Bioavailability from Environmental Media. Chlorobenzene is absorbed primarily following inhalation of contaminated air. There is also some potential for exposure from water and soil. Chlorobenzene has been detected at low levels in surface, ground, and drinking water, but no information was found on levels in food. Since chlorobenzene binds tightly to soil particles, skin contact with or ingestion of contaminated soil may be an important source of exposure, particularly in children living near hazardous waste sites. Additional studies would be useful to determine if soil-bound chlorobenzene is bioavailable.

Food Chain Bioaccumulation. No information is available regarding biomagnification within aquatic or terrestrial food chains. Additional studies would be useful in assessing potential for human exposure to chlorobenzene.

Exposure Levels in Environmental Media. There are studies on concentrations of chlorobenzene in air and water, but many of the samples measured had low levels or did not have detectable levels. Additional studies using more sensitive analytical methods would be useful.

Exposure Levels in Humans. Studies have been conducted measuring chlorobenzene levels in drinking water and air (including indoor air). Conflicting data on chlorobenzene air levels point to a need for confirmation and, possibly, validation of analytical methods. Less conflicting estimates of environmental emissions are the prerequisite for any attempt to prioritize control measures.

Exposure Registries. No exposure registries for chlorobenzene were located. This compound is not currently one of the compounds for which a subregistry has been established in the National Exposure Registry.

The compound will be considered in the future when chemical selection is made for subregistries to be established. The information that is amassed in the National Exposure Registry facilitates the epidemiological research needed to assess adverse health outcomes that may be related to the exposure to this compound.

5.7.2 On-going studies

Studies on the migration and in situ biodegradation of chlorobenzene in hazardous waste sites are being conducted in the laboratory of Perry McCarty and others.

As part of the Third National Health and Nutrition Evaluation Survey (NHANES III), the Environmental Health Laboratory Sciences Division of the Center for Environmental Health and Injury Control, Centers for Disease Control, will be analyzing human blood samples for chlorobenzene and other volatile organic compounds. These data will give an indication of the frequency of occurrence and background levels of these compounds in the general population.

6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting and/or measuring and monitoring chlorobenzene in environmental media and in biological samples. The intent is not to provide an exhaustive list of analytical methods that could be used to detect and quantify chlorobenzene. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used to detect chlorobenzene in environmental samples are methods approved by federal agencies such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by a trade association such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that refine previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

6.1 BIOLOGICAL MATERIALS

Many of the considerations regarding the analysis of halogenated alkanes and alkenes in biological samples (Fishbein 1985) similarly apply to the determination of chlorobenzene in these samples. Although most environmentally significant halogenated alkanes and alkenes have boiling points below 100°C, chlorobenzene is relatively less volatile with a boiling point of 132°C. The water solubility (25°C) of chlorobenzene is 472 mg/L, which is lower than the water solubilities of most environmentally and toxicologically significant halogenated alkanes and alkenes. Along with many halogenated alkanes and alkenes, chlorobenzene is classified as a purgeable species for purge-and-trap analysis (EPA 1982a, 1982b). Therefore, many of the approaches and methods used for the determination of halogenated alkanes and alkenes in biological samples are applicable to chlorobenzene, although they have not been validated as a sampling method.

Because chlorobenzene is volatile, has limited water solubility, and has a moderate affinity for lipid tissue, chlorobenzene is easily lost from biological samples. Appropriate care must be exercised in handling and storing such samples for analysis of chlorobenzene.

The methods that generally are used to remove volatile organic chemicals (VOCs) from biological samples for analysis are applicable to chlorobenzene. These include headspace analysis, purge-and-trap (gas stripping) collection from aqueous solutions or slurry samples, solvent extraction, and direct collection on resins. Headspace analysis offers speed, simplicity, and good reproducibility for a particular type of

sample. However, partitioning of the analyte between the headspace and the sample matrix is dependent upon the nature of the matrix and must be determined separately for different kinds of matrices (Walters 1986).

Purge-and-trap collection is well suited to biological samples that are soluble in water and is readily adapted to biological samples from techniques that have been developed for the analysis of halocarbons such as chlorobenzene in water and wastewater. For water-insoluble materials, the purge-and-trap approach is complicated by the uncertainty of partitioning the analyte between sample slurry particles and water.

Homogenization of tissue with the extractant and lysing of cells improves extraction efficiency. When multiple analytes are determined using solvent extraction, selective extraction and loss of low-boiling compounds can cause errors. The commercial availability of highly purified solvents has largely eliminated problems with solvent impurities, although high costs, solvent toxicities, and restrictions on spent solvent disposal must be considered. Directly coupled supercritical fluid extraction-gas chromatography has been used for the determination of polychlorinated biphenyls (Hawthorne 1988) and should work well for the determination of chlorobenzene in biological samples.

Analytical methods for the determination of chlorobenzene in biological samples are given in Table 6-1.

6.2 ENVIRONMENTAL SAMPLES

Purgeable organic compounds such as chlorobenzene can be determined in water by the purge-and-trap technique. This method consists of bubbling inert gas through a small volume of the sample and collecting the vapor in a trap packed with sorbent. The analytes are then removed from the trap by heating it and backflushing the analytes onto a gas chromatographic column. The two materials most widely used for adsorption and thermal desorption of volatile organic compounds collected by the purge-and-trap technique are Carbotrap® consisting of graphitized carbon black, and Tenax® a porous polymer of 2,6-diphenyl-pphenylene oxide (Fabbri et al. 1987).

TABLE 6-1. Analytical Methods for Determining Chlorobenzene in Biological Materials

Sample Matrix	Sample Preparation	Analytical Method	Sample Detection Limit	Accuracy	Reference
Breath, blood, urine	Breath collected on Tenax, blood and urine subjected to purge-and-trap, concentrated on cryogenic capillary trap, thermally removed to GC.	GC/MS	No data	No data	Barkley et al. 1980
Fish tissue	Grind with sodium sulfate, extract with hexane/acetone	GC/ECD	No data	No data	Oliver and Nicol 1982a, 1982b
Adipose tissue	Extraction, bulk lipid removal, Florisil fractionation	HRGC/MS	0.1 μg/g	No data	Mack and Stanley 1984
Adipose tissue	Heated dynamic headspace purge-and-trap	HRGC/MS	2 ng/g	No data	Stanley 1986
Biofluids[a]	Dilute with water, sealed vial, collection of headspace vapors	GC/ECD	No data	No data	Suitheimer et al. 1982
Blood, tissue	Macerate tissue in water, warm blood or tissue, pass inert gas through, trap on Tenax, thermal desorption	GC/MS	3 ng/mL blood 6 ng/g tissue	No data	Pellizzari et al. 1985

[a] Among the compounds for which this method was used are benzene, m-xylene, carbon tetrachloride and chloroform. The method can be adapted to chlorobenzene although the procedures do not list this compound specifically as an analyte.

GC = gas chromatography; MS = mass spectrometry; ECD = electron capture detector; HRGC = High Resolution Gas Chromatography; μg/g = microgram per gram; ng/g = nanogram per gram.

The introduction of capillary column chromatography has markedly improved both the sensitivity and resolution of gas chromatographic analysis of environmental samples such as chlorobenzene. Because of the very small quantities of sample required, capillary column chromatography has made sample delivery more difficult. One of the more promising approaches to sample introduction using capillary columns with purge-and-trap collection is the use of cryofocussing. Basically, this procedure consists of collecting purged analyte on a short section of the capillary column cooled to a low temperature (e.g., -100°C) temperature, followed by heating and backflushing of the sample onto the analytical column. Chlorobenzene has been determined in water by this method (Washall and Wampler 1988).

Chlorobenzene can be removed from water by adsorption on synthetic polymers contained in cartridges, followed by thermal desorption of analyte (Pankow et al. 1988). Among the products used for this purpose are Tenax-GC® and Tenax-TA®.

Analytical methods for the determination of chlorobenzene in environmental samples are given in Table 6-2.

6.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of chlorobenzene is available. Where adequate information is not available, ATSDR, in conjunction with the NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of chlorobenzene.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that, if met would reduce or eliminate the uncertainties of human health assessment. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

TABLE 6-2. Analytical Methods for Determining Chlorobenzene in Environmental Samples

Sample Matrix	Sample Preparation	Analytical Method	Sample Detection Limit	Accuracy	References
Air	Collect on Tenax GC, thermal desorption, cryogenic collection on a capillary trap, thermal transfer to GC	GC/MS	0.47 parts per trillion	No data	Krost et al. 1982
Air	Coconut shell charcoal sorption, carbon disulfide desorption	GC/FID	10 μg per sample	No data	NIOSH 1984
Water	Purge-and-trap	GC/HSD	0.25 μg/L	No data	EPA 1982a
Water	Purge-and-trap	GC/MS	0.2 μg/L	No data	EPA 1982b
Water	Purge-and-trap	GC/MS	6.0 μg/L	No data	EPA 1982c
Water	Sorption on small dead volume Tenax cartridges, thermal desorption	HRGC/MS	No data	No data	Pankow et al. 1988
Contaminated soil	Purge-and-trap	GC/HSD	300 μg/kg	No data	EPA 1986a
Wastes (non-water miscible) and soil	Purge-and-trap	GC/MS	250 μg/kg	No data	EPA 1986b
Wastes (water miscible and non-water miscible) and soil	Purge-and-trap	GC/MS	250-2500 μg/kg	No data	EPA 1986c

GC = gas chromatography; MS = mass spectrometry; FID = flame ionization detector; μg = microgram; HSD = halide specific detector; L = liter; HRGC = high resolution gas chromatography; kg = kilogram.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. Excellent sensitive and selective methods are available for the qualitative and quantitative measurement of the parent compound, chlorobenzene after it is separated from its sample matrix. Methods need to be validated for chlorobenzene.

Further studies on the transfer analytes that have been purged or extracted from a biological or environmental sample quantitatively and in a narrow band to the capillary GC would better characterize exposure. Improvements in cryofocussing of VOC analytes for capillary GC determination of VOCs (Washall and Wampler 1988) should improve sensitivity for the determination of chlorobenzene.

Metabolites of chlorobenzene in biological materials cannot be determined in routine practice because of the lack of standard methods for measuring these metabolites. Further research on supercritical fluid (SCF) extraction holds great promise for meeting the goals of quantitative, rapid, easily performed, low cost, and safe procedures for the determination of nonpolar organic analytes such as chlorobenzene in biological samples.

Central nervous system, liver, and kidney injuries are characteristic biomarkers for effects of chlorobenzene intoxication. Since the effects are indicative of exposure to many other toxicants, methods are needed for more specific biomarkers.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods for determining the parent compound, chlorobenzene, in water, air, and waste samples with excellent selectivity and sensitivity are highly developed, thus the database in this area is good and undergoing constant improvement.

Means to measure organohalides such as chlorobenzene in situ in water and other environmental media could contribute to environmental studies of this compound.

Degradation products of chlorobenzene in environmental media are difficult to determine. This difficulty is not so much an analytical problem as it is a problem of knowing the fundamental environmental chemistry of these compounds in water, soil, air, and biological systems.

6.3.2 On-going Studies

Research is ongoing to develop a "Master Analytical Scheme" for organic compounds in water (Michael et al. 1988), which includes chlorobenzene as an analyte. The overall goal is to detect and quantitatively measure organic compounds at 0.1 μg/L in drinking water, 1 μg/L in surface waters, and 10 μg/L in effluent waters. Analytes are to include numerous semivolatile compounds and some compounds that are only "semi-soluble" in water, as well as volatile compounds (bp < 150°C).

The Environmental Health Laboratory Sciences Division of the Center for Environmental Health and Injury Control, Centers for Disease Control, is developing methods for the analysis of chlorobenzene and other volatile organic compounds in blood. These methods use purge and trap and magnetic mass sector spectrometry which gives detection limits in the low parts per trillion range.

7. REGULATIONS AND ADVISORIES

Because of its potential to cause adverse health effects in exposed people, a number of regulations and advisories have been established for chlorobenzene by various national and state agencies. These values are summarized in Table 7-1.

7. REGULATIONS AND ADVISORIES

TABLE 7-1. Regulations and Guidelines Applicable to Chlorobenzene

Agency	Description	Value	Reference
	National		
Regulations:			
a. Air:			
OSHA	PEL TWA	75 ppm (350 mg/m^3)	OSHA 1989 (29 CFR 1910.1000, Table Z-1-A)
b. Water:			
EPA ODW	Monitoring required for unregulated contaminants	NA	EPA 1987a, (40 CFR 141.40)
	MCL (Proposed)	0.1 mg/L	EPA 1989c
EPA OWRS	General permits under NPDES	NA	40 CFR 122, (Appendix D, Table II)
	Criteria and Standards for the NPDES	NA	40 CFR 125
	General pretreatment regulations for existing and new sources of pollution	NA	40 CFR 403
	Hazardous substance	NA	EPA 1985b, (40 CFR 116)
	Reportable quantity	100 lb	40 CFR 117.3
c. Nonspecific media:			
EPA OERR	Reportable quantity	100 lb	EPA 1985b, (40 CFR 302.4)
EPA OSW	Hazardous waste constituent (Appendix VIII)	NA	EPA 1980b, (40 CFR 261)
	Groundwater monitoring list (Appendix IX)	NA	EPA 1987b, (40 CFR 264)
	Restriction on land disposal	NA	EPA 1988b, 1989c, (40 CFR 268)
EPA OTS	Preliminary assessment information rule	NA	EPA 1982d, (40 CFR 712)
	Health and safety data reporting rule	NA	EPA 1988c, (40 CFR 716.120)
	Final test rule	NA	EPA 1986e, (40 CFR 799.105)
	Toxic chemical release reporting	NA	EPA 1988c, (40 CFR 372)
Guidelines:			
a. Air:			
ACGIH	TLV TWA	75 ppm (350 mg/m^3)	ACGIH 1986
NIOSH	IDLH	2400 ppm	NIOSH 1985
b. Water:			
EPA ODW	MCLG (proposed)	0.1 mg/L	EPA 1989c
	Health advisories		
	1 day	2 mg/L	EPA 1987c
	10 days	2 mg/L	

TABLE 7-1 - (Continued)

Agency	Description	Value	Reference
	Longer term		
	child	2 mg/L	
	adult	7 mg/L	
	Lifetime	100 mg/L	
EPA OWRS	Ambient water quality criteria		
	Ingesting water and organisms	4.88×10^{-1} mg/L	EPA 1980b
c. Other:			
EPA	Carcinogenic classification	Group D[a]	EPA 1987c
	Oral RfD	2×10^{-2} mg/kg/day	IRIS 1989
	State		
Regulations:			
a. Air:	Acceptable ambient air concentration		NATICH 1988
Connecticut		7000 μg/m^3 (8 hr)	
Florida-Tampa		3500 μg/m^3 (8 hr)	
Massachusetts		6.3 μg/m^3 (24 hr)	
Nevada		8.333 μg/m^3 (8 hr)	
New York		1167.0 μg/m^3 (1 yr)	
North Carolina		2200 μg/m^3 (24 hr)	
North Dakota		3500 μg/m^3 (8 hr)	
Virginia		6000 μg/m^3 (24 hr)	
b. Water:	Drinking water		FSTRAC 1988
Arizona		60 μg/L	
California		30 μg/L	
Kansas		60 μg/L	
Maine		47 μg/L	
Minnesota		60 μg/L	
New Jersey		2 μg/L	
Vermont		600 μg/L	
Wisconsin		600 μg/L	

[a] Group D: Not classifiable as to human carcinogenicity: Inadequate human and animal evidence of carcinogenicity.

OSHA = Occupational Safety and Health Administration; PEL = Permissible Exposure Limit; TWA = Time-Weighted Average; EPA = Environmental Protection Agency; ODW = Office of Drinking Water; NA = Not Applicable; MCL = Maximum Contaminant Level; OWRS = Office of Water Regulations and Standards; NPDES = National Pollutant Discharge Elimination System; OERR = Office of Emergency and Remedial Response; OSW = Office of Solid Wastes; OTS = Office of Toxic Substances; ACGIH = American Conference of Governmental Industrial Hygienists; TLV = Threshold Limit Value; NIOSH = National Institute for Occupational Safety and Health; IDLH = Immediately Dangerous to Life or Health Level; MCLG = Maximum Contaminant Level Goal; RfD = Reference dose.

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9. GLOSSARY

Acute Exposure -- Exposure to a chemical for a duration of 14 days or less, as specified in the Toxicological Profiles.

Adsorption Coefficient (K_{oc}) -- The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

Adsorption Ratio (Kd) -- The amount of a chemical adsorbed by a sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

Bioconcentration Factor (BCF) -- The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same time period.

Cancer Effect Level (CEL) -- The lowest dose of chemical in a study or group of studies which produces significant increases in incidence of cancer (or tumors) between the exposed population and its appropriate control.

Carcinogen -- A chemical capable of inducing cancer.

Ceiling value (CL) -- A concentration of a substance that should not be exceeded, even instantaneously.

Chronic Exposure -- Exposure to a chemical for 365 days or more, as specified in the Toxicological Profiles.

Developmental Toxicity -- The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Embryotoxicity and Fetotoxicity -- Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the insult occurred. The terms, as used here, include malformations and variations, altered growth, and in utero death.

9. GLOSSARY

EPA Health Advisory -- An estimate of acceptable drinking water levels for a chemical substance based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

Immediately Dangerous to Life or Health (IDLH) -- The maximum environmental concentration of a contaminant from which one could escape within 30 min without any escape-impairing symptoms or irreversible health effects,

Intermediate Exposure -- Exposure to a chemical for a duration of 15-364 days, as specified in the Toxicological Profiles.

Immunologic Toxicity -- The occurrence of adverse effects on the immune system that may result from exposure to environmental agents such as chemicals.

In Vitro -- Isolated from the living organism and artificially maintained, as in a test tube.

In Vivo -- Occurring within the living organism.

Lethal Concentration($_{LO}$) (LC_{LO}) -- The lowest concentration of a chemical in air which has been reported to have caused death in humans or animals.

Lethal Concentration($_{50}$) (LC_{50}) -- A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose($_{LO}$) (LD_{LO}) -- The lowest dose of a chemical introduced by a route other than inhalation that is expected to have caused death in humans or animals.

Lethal Dose($_{50}$) (LD_{50}) -- The dose of a chemical which has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time($_{50}$)(LT_{50}) -- A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL) -- The lowest dose of chemical in a study or group of studies which produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

9. GLOSSARY

Malformations -- Permanent structural changes that may adversely affect survival, development, or function.

Minimal Risk Level (MRL) -- An estimate of daily human exposure to a chemical that is likely to be without an appreciable risk of deleterious effects (noncancerous) over a specified duration of exposure.

Mutagen -- A substance that causes mutations. A mutation is a change in the genetic material in a body cell. Mutations can lead to birth defects, miscarriages, or cancer.

Neurotoxicity -- The occurrence of adverse effects on the nervous system following exposure to a chemical.

No-Observed-Adverse-Effect Level (NOAEL) -- That dose of chemical at which there are no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Effects may be produced at this dose, but they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{ow}) -- The equilibrium ratio of the concentrations of a chemical in n-octanol and water, in dilute solution.

Permissible Exposure Limit (PEL) -- An allowable exposure level in workplace air averaged over an 8-hour shift.

q_1* -- The upper-bound estimate of the low-dose slope of the doseresponse curve as determined by the multistage procedure. The q_1* can be used to calculate an estimate of carcinogenic potency, the incremental excess cancer risk per unit of exposure (usually μg/L for water, mg/kg/day for food, and μg/m^3 for air).

Reference Dose (RfD) -- An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure of the human population to a potential hazard that is likely to be without risk of deleterious effects during a lifetime. The RfD is operationally derived from the NOAEL (from animal and human studies) by a consistent application of uncertainty factors that reflect various types of data used to estimate RfDs and an additional modifying factor, which is based on a professional judgment of the entire database on the chemical. The RfDs are not applicable to nonthreshold effects such as cancer.

Reportable Quantity (RQ) -- The quantity of a hazardous substance that is considered reportable under CERCLA. Reportable quantities are: (1) 1 lb or greater or (2) for selected substances, an amount established by

regulation either under CERCLA or under Sect. 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

Reproductive Toxicity -- The occurrence of adverse effects on the reproductive system that may result from exposure to a chemical. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

Short-Term Exposure Limit (STEL) -- The maximum concentration to which workers can be exposed for up to 15 min continually. No more than four excursions are allowed per day, and there must be at least 60 min between exposure periods. The daily TLV-TWA may not be exceeded.

Target Organ Toxicity -- This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen -- A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV) -- A concentration of a substance to which most workers can be exposed without adverse effect. The TLV may be expressed as a TWA, as a STEL, or as a CL.

Time-weighted Average (TWA) -- An allowable exposure concentration averaged over a normal 8-hour workday or 40-hour workweek.

Toxic Dose (TD_{50}) -- A calculated dose of a chemical, introduced by a route other than inhalation, which is expected to cause a specific toxic effect in 50% of a defined experimental animal population.

Uncertainty Factor (UF) -- A factor used in operationally deriving the RfD from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of humans, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using LOAEL data rather than NOAEL data. Usually each of these factors is set equal to 10.

APPENDIX

PEER REVIEW

A peer review panel was assembled for chlorobenzene. The panel consisted of the following members: Dr. David Jollow, Professor in the Department of Pharmacology, Medical University of South Carolina, Charleston, South Carolina; Dr. Henry Peters, Professor in the Department of Neurology, University of Wisconsin Clinical Science Center, Madison, Wisconsin; Dr. Jay B. Silkworth, Research Scientist, Wadsworth Center Labs, New York Department of Health, Albany, New York; Dr. Frank Lu, Private Toxicology Consultant, Miami, Florida; Dr. James Pollard, Private Consultant, Las Vegas, Nevada. These experts collectively have knowledge of chlorobenzene's physical and chemical properties, toxicokinetics, key health end points, mechanisms of action, human and animal exposure, and quantification of risk to humans. All reviewers were selected in conformity with the conditions for peer review specified in Section 104(i)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

A joint panel of scientists from ATSDR and EPA has reviewed the peer reviewers' comments and determined which comments will be included in the profile. A listing of the peer reviewers' comments not incorporated in the profile, with a brief explanation of the rationale for their exclusion, exists as part of the administrative record for this compound. A list of databases reviewed and a list of unpublished documents cited are also included in the administrative record.

The citation of the peer review panel should not be understood to imply their approval of the profile's final content. The responsibility for the content of this profile lies with the Agency for Toxic Substances and Disease Registry.