UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

April 10, 1985

Honorable Lee M. Thomas
Administrator
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460

OFFICE OF
THE ADMINISTRATOR

Dear Mr. Thomas:

The Environmental Health Committee of EPA's Science Advisory Board has completed its review of the Agency's draft Health Assessment Document for Chloroform. The stated purposes of the document are to serve as a source document for Agencywide use and to serve as a scientific basis for decision making on hazardous air pollutants by the Office of Air and Radiation.

The Committee found the Chloroform document to be of better overall scientific quality than draft health assessment documents for other substances that the Committee has reviewed during the past year. In addition, the Committee appreciated the responsiveness of Agency scientists in responding to its questions during the review. The Committee agrees with the conclusions stated in the document that: (1) chronic exposure to Chloroform is associated with renal, cardiac, neurological and hepatotoxic effects, and (2) sufficient pharmacokinetic data exist for Chloroform to incorporate this information in the quantitative risk estimates. Before the final version is printed, the Committee requests that the Agency perform additional work to improve the document in three areas. These include carcinogenicity, mutagenicity and teratology. Detailed comments on these and other issues are provided in the attached technical report. In summary, the Committee concludes that the Health Assessment Document for Chloroform is scientifically adequate for its stated purposes. Unless the Agency requests additional advice from the Committee, the current draft should not require further review.

We appreciate the opportunity to comment on this public health issue and stand ready to provide any further scientific advice. We request a written response to our advice.

Sincerely,

Richard A. Griesemer
Richard A. Griesemer, D.V.M., Ph.D.
Chair, Environmental Health Committee

Norton Nelson
Norton Nelson, Ph.D.
Chair, Executive Committee

Enclosure

REPORT OF THE ENVIRONMENTAL HEALTH COMMITTEE OF EPA'S SCIENCE ADVISORY BOARD REGARDING A DRAFT HEALTH ASSESSMENT DOCUMENT FOR CHLOROFORM

INTRODUCTION

On December 20, 1984, the Chlorinated Organics Subcommittee of the Environmental Health Committee reviewed a draft Health Assessment Document for Chloroform [EPA-600/ 8-84-0004A; March, 1984; External Review Draft]. The document was prepared by the Office of Health and Environmental Assessment (OHEA) in the Office of Research and Development. The Subcommittee report, signed by the Chair, Dr. John Doull, is separately available. Subsequently, the draft document was reviewed by the full Committee. The Committee's major conclusions and technical comments are presented below.

The draft document, including revisions proposed by OHEA staff, generally was well-written, in that:

(1) the relevant literature was analyzed critically,

(2) interpretation of the literature was organized about a central point of view,

(3) the document exhibited an open-minded approach to the data,

(4) the different chapters reached scientifically reasonable conclusions, and

(5) an effort was made to interrelate the results of different studies within chapters and to integrate the conclusions of different chapters.

In addition, the scientists in charge of revising different portions of the draft document came to the meeting prepared to comment on advice about closely related issues from other Committee reviews. Therefore, a productive dialogue ensued.

MAJOR COMMENTS

The Committee requests that OHEA scientists do further concerted work on three chapters: carcinogenicity, mutagenicity and teratology. The primary reasons for the Committee's requests are discussed in this section. More detailed comments are discussed in the appropriate sections below. These comments should not be misconstrued as indicating a desire by the Committee to review the draft further.

Using the criteria developed by the International Agency for Research on Cancer (IARC), the document concludes that sufficient animal evidence of the carcinogenicity of chloroform exists, but that the epidemiologic evidence is inadequate. Consistent with the IARC criteria, chloroform would be placed into Category 2B, meaning that it is probably carcinogenic to humans. The Committee agrees with the conclusion regarding the animal evidence, but requests that OHEA staff further analyze the conclusions regarding epidemiologic evidence. The Committee understands that the dividing line between the IARC definitions of "inadequate" and "limited" evidence

is a fine one. However, the criterion of limited evidence is that "a causal explanation is credible," and the evidence regarding chlorinated drinking water may appear to meet this standard.

The Committee agrees with the statement in the Executive Summary that chloroform is a potential inhalational teratogen. However, the supporting text interprets only teratogenic effects. The corresponding evidence for a fetotoxic effect of chloroform is described but not evaluated. The Committee suggests that OHEA analyze both teratogenicity and fetotoxicity data for levels at which no effects are observed.

The Committee believes that the conclusion that chloroform may be a weak mutagen is not well documented. It is equally plausible that this substance is a nonmutagen and that false positive results have been obtained in a few tests. While more or newer tests might detect mutagenic activity at lower concentrations, current testing methods provide the state-of--the-art definition of a mutagen.

EXPOSURE

For the Committee's background information, the Office of Air Quality Planning and Standards provided a written "Summary of Exposure Information for Chloroform (Trichloromethane)", which was helpful in evaluating the health effects information in the draft document. The health assessment document also presents information regarding the atmospheric chemistry of chloroform, which lies beyond the expertise of the current members of the Committee to review. The Committee understands, however, that OHEA has obtained expert peer review of this section elsewhere.

PHARMACOKINETICS

Data exist on the pharmacokinetics of chloroform in three mouse strains, as well as rat, monkey and man. These data suggest that chloroform is rapidly and completely absorbed. The unchanged portion of chloroform principally is excreted through the lung. No evidence exists for different metabolic pathways. Therefore, the Committee suggests that OHEA can reasonably assume that in different species the qualitative metabolic pathways for metabolism do not differ among mammalian species.

Chloroform metabolism is saturated (or close to saturated) at bioassay doses. This implies that correction to internal dose will affect the slope of potency estimates. Chloroform is metabolized to reactive intermediates, which will bind covalently to cellular macromolecules. Evidence is consistent with an increase in covalent binding to a point of saturation. One reactive metabolite, phosgene, is a causative factor in the mechanism of renal and hepatic toxic effects of chloroform.

The time period of 7 to 8 days (cited on page 3 of the Carcinogen Assessment Group's "Replies to Public Comments" on the draft document, dated October 10, 1984) will appply at any given exposure, due to the nature of first order kinetics. The Committee is curious whether any additional data exist regarding the kinetics of elimination at different exposures. If the kinetics are biexponential, then unusual kinetic

phenonema can occur, but the data suggest a one compartment model for chloroform. Thus, acute versus chronic exposure will not change the elimination rate. The Committee also suggests that the chapter on pharmacokinetics illustrate glucuronide formation in Figure 4-6 (see page 4-36).

OHEA should correct the carbon monoxide-anaerobic pathway, as discussed at the meeting. Metabolism of chloroform to phosgene may not be the only active product involved. Electrophilic halogens also may be produced. The February 1984 issue of Trends in Pharmacological Sciences contains an article on this subject.†

GENERAL TOXICOLOGY

The acute toxicity section is well-written and is a scientifically defensible statement of the literature on this substance. The Committee agrees with the conclusions reached that chronic exposure to specific concentrations of chloroform elicts renal, cardic, neurological and hepatotoxic effects.

MUTAGENICITY

In addition to the draft health assessment document, the Committee received a revised chapter on mutagenicity prior to the meeting from OHEA's Reproductive Effects Assessment Group. The Committee believes that this chapter attempts to interpret critically an extensive and somewhat contradictory data base.

The revised chapter concludes that the available information supports the finding that chloroform may be a "weak" (or low potency) mutagen. The Committee does not agree with this finding and suggests instead that it is possible that extensive testing for mutagenicity of chloroform may have generated some false positive results with a nonmutagen. The conclusions regarding mutagenicity of chloroform do not appear to reflect in full the weight of the evidence and need further amplification, in part because new and unproven test methods were applied to elicit some weakly positive results. Within the scientific community the current working definition of a "mutagen" depends on test results obtained with standardized protocols. While the evaporation of chloroform may result in negative results with some procedures, all of the tests with which the chapter appears to have interpretational difficulty also have negative results. These negative results come from reliable methods, whereas the interpretational difficulty comes from a bias towards positive results.

We request that OHEA reexamine the testing evidence from the point of view that the hypothesis of false positive results needs to be refuted and that the validity of the positive evidence needs to be investigated equally with the negative.

A recent book by DeSerres and Ashby describes results from thirty-

---

† L.R. POHL and B.A. MICO, Electrophillic Halogens as Potentially Toxic Metabolites of Halogenated Compounds. Trends in Pharmacological Sci.

seven highly qualified laboratories which have conducted short-term testing on chloroform. These findings are discounted in the document. Seven of forty studies in this book were positive (one of six DNA repair tests, two of nineteen bacterial mutation tests, four of thirteen eukaryote tests, and neither of the two whole animal tests). These tests were performed with well-defined, standardized protocols, and the results futher emphasize the possibility of false positive results with a nonmutagen.

"Insensitivity" in the DeSerres and Ashby reference was defined with respect to the detection of a potential carcinogen, not a mutagen. When insensitivity is discussed in the draft health assessment document, however, the reference is to detection of a potential mutagen. Where the chapter on mutagenicity of chloroform describes the conclusions from DeSerres and Ashby book, the definition of a false negative result (in relation to a potential carcinogen) should be used.

The conclusion regarding the mutagenicity of chloroform has important implications for the qualitative findings for carcinogenicity. If chloroform is a not a mutagen but rather a carcinogen, some scientists will treat chloroform as a presumptive promoter, although some Committee members question the adequacy of mutagenic tests to conclude that chloroform is a "promoter". The Committee requests that the final document further discuss this issue in detail.

The Committee also advises that the use of qualifiers such as "weak" and "strong" to describe mutagens is potentially misleading, because potency can be confused with either the qualitative weight of evidence for mutagenic effects or the severity of mutational effect. Use of the terminology suggested for weight-of-the-evidence categories in the Agency's proposed guidelines for mutagenicity risk assessment may partially alleviate this problem. Perhaps potency can be described better through reference to other agents or to the actual quantitative information.

Discussions of the papers by Direnzo and by Castro are inconsistent with each other. The Castro study had the same specific activity ^{14}C-labelled chloroform as the Direnzo study. Therefore, specific activity of the isotope cannot be an adequate basis to dismiss one study and accept the other. In addition, the numerical results reported are in disagreement between the two studies and require further comment.

Inconsistency also occurs in the discussion of microsomal preparations in these two papers. Castro used mouse microsomes and found no DNA binding, whereas Direnzo used rat microsomes and found DNA binding. The document should carefully and consistently implement the conclusions regarding incorporation of chloroform into DNA in all chapters. The Castro study consists of two papers, and the data from each paper needs to be interpreted in the light of the other. Castro found lipid and protein binding in one paper. This binding shows that activation of chloroform took place with mouse microsomal preparations.

CARCINOGENICITY

The staff of OHEA's Carcinogen Assessment Group distributed a revised Carcinogenicity chapter at the meeting on which some of their oral comments

were based. Much of the discussion below reflects the oral presentation made by OHEA scientists at the Chlorinated Organics Subcommittee meeting.

Neither the Subcommittee nor the Committee has had the resources then (or subsequently) to review the revised chapter in detail or to collate the revised chapter with the oral presentation. Reference to the oral presentation may be obtained through the transcript of the meeting, which is available through EPA's Committee Management Office [PM-213, Room 2515 Mall]. However, the Committee regards the changes suggested by staff favorably and desires to see them implemented in the final version of this chapter.

The Committee agrees with the qualitative conclusion in the document that sufficient animal evidence of the carcinogenicity of chloroform exists from replicated studies. The animal studies are histologically and biologically sound, and they have been adequately interpreted. According to IARC criteria, this information will place chloroform into Category 2, which is defined as "the chemical... probably is carcinogenic to humans." However, as described above under "Major Comments," the Committee questions whether the human evidence is inadequate or limited. Therefore, the Committee requests that OHEA's scientists reexamine the epidemiological evidence regarding carcinogenic effects of chlorinated drinking water, to confirm or modify the conclusion regarding the weight-of-the-evidence for humans in the light of the comments below.

The Committee believes that the terminology used by IARC is "inadequate" evidence, not "insufficent" evidence. (See page 1-10 of the draft document.) The criteria of inadequate versus limited evidence are very close together, and the Committee appreciates the difficulty facing OHEA's scientists in applying these definitions in the case of chloroform in drinking water. However, the criterion of "limited" evidence is that " a causal explanation is credible." The comparison of the quantitative estimates of risk based on animal versus human information suggests that the animal and human data are not inconsistent, although the quantitative estimates are highly uncertain. (See "Quantitative Estimates of Risk" below.) The Committee looks at the evidence as follows:

(1) The statistical power of any of of the individual studies is weak. The ratios range from 1.1 to 2.0. The middle of this range, however, represents a 50% increase in the incidence of common human cancers, which is substantial.

(2) The specificity of the cancers found in the different studies are identical; that is, drinking chlorinated water is associated consistently with cancers of the rectum, bladder and colon.

(3) The association between chloroform in drinking water and human cancers has biological plausiblity. The route of ingestion and excretion is rationally related to the organs with which cancer is associated. Because chloroform causes tumors in animals, it is reasonably suspect in the human surveys.

(4) No dose-response relationship was found.

(5) The different studies are consistent with each other. A positive association with the same cancers was found in all studies.

While a cautious interpretation is warrented, it will be worthwhile to reexamine all of the data in the light of the IARC criteria. Technically, the Committee suggests that OHEA try the method of residues. The data on leukemias could be extracted from the existing data, and the residues reanalyzed for evidence of associations.

Once OHEA has reinterpreted the epidemiological evidence for chloroform, the Committee further requests some editing of the draft, regardless of the qualitative conclusion. The analyses of individual studies of chlorinated drinking water each close with strongly worded summaries. These summaries appear inconsistent with the overall summary of the human evidence. The utility of the fifth paragraph on page 8-36 is questionable. The logic fails in many analogies. For example, dioxins are minor contaminants of Agent Orange, but they are thought responsible for most of the toxic properties of this pesticide.

A new study of chloroform carcinogenicity in animals is forthcoming, which was conducted by SRI International. A review of this study could be added to the draft document or later placed into an addendum. The results of this bioassay were not reviewed in detail by the Subcommittee; they could affect the quantitative conclusions but are not likely to change the qualitative findings. The evidence from this and other studies of chloroform tends to pinpoint some general issues about bioassays. These issues reflect the state-of-the-art in toxicology, and it may be useful to the Agency to comment on them in the context of the new study.

In the SRI bioassay, no response was obtained when the C57B16 mouse was given chloroform in drinking water, while chloroform administered in corn oil yielded positive results. Thus, the risk of cancer in mice cannot be related to chloroform alone in this assay. In contrast, approximately the same results were obtained with both vehicles in the rat.

The SRI results raise two significant issues. First, complete absorption occurs with both vehicles. Absorption is faster when chloroform is administered in drinking water, but the animals sip water throughout the day. Therefore, the vehicle dependence of the findings can not be explained by incomplete absorption with water in comparison to corn oil. Second, a bolus effect occurs with corn oil, since corn oil is employed in gavage studies. A pharmacokinetic analysis may be required to explain the effects of vehicle on the carcinogenicity of chloroform for the mouse.

A discussion of the issue of liver damage in relation to carcinogenicity will improve the chapter. The issue of liver damage relates to the possibility of chloroform acting as a carcinogen through promoter effects. OHEA scientists discussed how the experimental data support several possible mechanisms of carcinogenesis. While this information is conjectural, the Committee believes it will improve the document to discuss the possible mechanisms in the chapter. The issue also is important in relation to bolus effects.

The Committee thinks that an ideal study of carcinogenicity will have evidence for or against the presence of tumors at doses below a maximally tolerated dose for non-carcinogenic toxic effects. The difficulty with this ideal approach is that dose-related toxicity studies do not often yield a clear-cut indication of the maximally tolerated dose. It will be useful to discuss this problem in the document within the context of the available chloroform bioassays, since there is a growing awareness in the scientific community of this problem with bioassays.

The question of a correlation between hepatotoxicity and hepatic carcinogenesis is of particular interest. The same mechanism probably is involved in the early stages of both toxic effects. Therefore, a correlation between the two endpoints is expected. Perhaps it is more unusual why chloroform is not more carcinogenic. The hepatotoxic and hepatic carcinogenic effects of chloroform can not be totally separated, as the data on page 8-19 show. After liver necrosis has occurred, if chloroform administration is stopped, then tumor formation also stops. Emanuel Farber has described a model of hepatic carcinogenesis through toxic effects on different populations of liver cells. His model may be of use to OHEA.

While the Committee recommends the addition of competing theories of chloroform-induced cancer to the discussion, these optional mechanisms should relate specifically to chloroform. We recommend that "boilerplate" language not required for the analysis of chloroform be removed from the text.

TERATOGENICITY AND FETOTOXICITY

Prior to its meeting, the Committee received a revised chapter on teratogenicity and reproductive effects from the Reproductive Effects Assessment Group. Even with the proposed revisions, the chapter on teratogenicity and reproductive effects merits further changes.

There are a number of reasons for this recommendation. The evidence for a fetotoxic effect of chloroform is described but not evaluated in the text. With the existing data for inhalational and ingestational routes of administration, the Committee believes that it is possible to derive no-observed-effect-levels for teratogenic effects of chloroform but that no evidence exists for either route of administration that levels have been found at which fetotoxic effects do not occur. There is no conclusion in the Executive Summary regarding fetotoxic effects of chloroform. The discussion in the revised chapter is focused only on teratogenic effects. The Committee suggests that OHEA analyze the levels at which no effects are observed for both of the endpoints, teratogenicity and fetotoxicity.

The Committee agrees with the statement in the Executive Summary that chloroform is a potential inhalational teratogen. However, the discussion in the chapter on teratology partially contradicts this finding. In addition, a number of technical errors in the chapter should be revised. The Committee has supplied detailed comments on this subject directly to OHEA.

QUANTITATIVE ESTIMATES OF RISK

During the discussion with the Committee, OHEA scientists suggested the possibility of conducting a risk assessment for teratogenic or fetotoxic effects of chloroform. The Committee agrees that this subject is worthy of pursuit. Fetotoxic effects apparently occur at doses similar to those that elicit carcinogenicity but with much shorter exposures. The Committee believes, however, that it is not reasonable to extrapolate to low doses and expect a proportionally low incidence of terata or lost conceptuses. The concept of a threshold for teratogenic effects is based on the capacity of the developing fetus to repair damage. A "one-hit" assumption is not reasonable.

Scientists from the Carcinogen Assessment Group also addressed the problem of scaling the absorbed dose between species. Sufficient data exist regarding the allometric relationship for chloroform to use a correction factor to obtain human equivalent doses (not necessarily human equivalent responses). The Committee believes that the approach taken in these calculations is appropriate, and that it responds to concerns the Science Advisory Board has raised with the hazard assessments of other substances.

A calculation also was orally presented that explored the question of sensitivity of response to dose units. The Committee felt particularly gratified to inspect this work, since it responds to recommendations made by the Environmental Health Committee in its review of previous health assessment documents. The Committee found the approach taken in these calculations to be scientifically reasonable. In addition, they tend to make explicit the information needed to understand the significance of the assumptions used in deriving the unit risk factor.

The Committee suggests that the term "metabolized dose" instead of "body burden" be used in the discussion, to prevent confusion with the concept of amounts of chemical accumulated on chronic administration. If a correction for metabolized dose is made, then new potency estimates are found which do not differ much from the previous, uncorrected estimate for chloroform. This calculation shows that the potency estimate for chloroform is not sensitive to assumptions regarding metabolized dose. Because data are available for only one dose for this calculation, the Committee finds it impossible to comment on the question of nonlinearity of response with metabolized dose.

Carcinogen Assessment Group staff also compared the potency estimate derived from animal data to the available epidemiology information, by means of a "what-if" calculation. Given appropriate caveats, the Committee believes that the display of the calculation is a good way to illustrate some of the uncertainties and consequences of the potency estimates for decision-makers. Studies of the association between drinking water and cancer incidence yield a range of relative risks. Chloroform, thought to arise from the chlorination of organic material in drinking water, was present in varying concentrations in these studies.

Given some assumptions, the "what-if" calculation shows that the uncertainty in the unit risk estimate might be of three to fours orders of magnitude (1,000-10,000). These calculations are the same in principle as estimates prepared by the National Research Council's Committee on the Biological Effects of Ionizing Radiation. The Committee also suggests that it would be reasonable to illustrate an hypothesis derived by back-calculating from the U.S. incidence of bladder cancer, assuming that all bladder cancer is due to the presence of chloroform in water. This calculation could also be treated as a doubling dose calculation.

The terminology by which the unit risk estimate is implemented is inconsistent in the draft document. For example, on page 8-79 the upper bound nature of the estimate is not stated accurately.

RANKING OF RELATIVE POTENCIES

OHEA has modified the table (pages 8-82 to 84) of relative potencies from previous documents to include a new column that lists IARC categories of the substances under comparison. The accompanying histogram (page 8-81) that illustrates the relative potencies of substances previously reviewed by the Carcinogen Assessment Group has not been changed and does not incorporate IARC categories. The Committee does not believe that insertion of IARC categories removes the concerns, as expressed in previous reviews of other health assessment documents, regarding the potential confusion between potency and severity. The problem is no different in principle than the distinction made between potency and efficacy with pharmacological agents. Describing only potency overemphasizes the lowest dose that might have an effect.

If it is crucially important to retain the table, the Committee suggests that OHEA also add columns for factors such as:

(1) the fraction of the maximally tolerated dose (or the dose of some other indicator of toxicity) at which a carcinogenic response is seen,

(2) the percent of animals which had tumors at the dose at which maximum tumor incidence was observed,

(3) the number of species in which tumors were observed, and

(4) the degree of malignancy of the tumors observed.

RESEARCH STUDIES

The draft document calls for additional research studies in several areas, particularly epidemiology. The Committee agrees that the proposals probably will improve our knowledge, but the proposals would benefit from greater explanatory detail in the text. Are some of the projects key studies? More extensive descriptions would have a value beyond the Agency's immediate needs.

LITERATURE REVIEW

The Committee requests that all future health assessment documents identify the date at which the literature review was completed. Only citations which substantially affect critical issues should be introduced beyond this date (perhaps in an addendum). Some of the references appear to need an update. For example, some references on metabolism are cited as "in press" or "submitted" in 1982. Further, the Committee requests that future documents describe the general nature of the information cited, such as peer reviewed articles, primary data from industry-sponsored toxicity studies, and so forth.