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Possible TSCA 8(e) Submission

Please find enclosed a copy of the study entitled "*Evaluation of the Respiratory Effects From Components of a Metalworking Fluid in Mice*". This study is being sent for possible submission under Section 8(e) of the Toxic Substances Control Act (TSCA).

One of the components tested in this acute inhalation mouse bioassay was a triazine biocide (CAS #4719-04-4: 1,3,5-triazine-1,3,5-(2H, 4H, 6H)-triethanol). The experimental protocol involved single, 180-minute exposures of four mice per dose to an undiluted aerosol of triazine at exposure concentrations (determined gravimetrically) of 55, 103, 204, 332 and 1,619 mg/m³. Animals were observed for seven days post exposure.

Mortality was observed in one of four animals at the 55 mg/m³ exposure concentration; one of three at 103 mg/m³; two of four at 204 mg/m³; two of four at 332 mg/m³; and two of three at 1,619 mg/m³ (see Table 7).

It should be noted that there was significant disparity between the analytically and nominally determined exposure concentrations (approximately 4-fold difference - see Table 5). This disparity was due to the vapor pressure of triazine (15 mm/Hg). The analytical method employed (gravimetric analysis) to determine aerosol concentrations is inappropriate for compounds with significant vapor pressures. The actual triazine exposure concentrations produced in this study would be higher than those measured. Animals would have been simultaneously exposed to both triazine aerosol and vapor, the vapor concentrations not being reflected in the gravimetric concentrations reported in Table 5. In addition due to evaporative losses from the filters used for this analysis, the aerosol concentrations would also have been underestimated (Page 35).

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Given the high triazine aerosol/vapor concentrations employed in this study (i.e., nominal concentration estimates suggest actual exposure concentrations may have ranged from approximately 200 to 6,000 mg/m³) and the known irritation and sensitization potential of triazines, the mortality results observed would be anticipated.

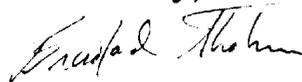
Triazine is a formaldehyde condensate biocide with a mode of action involving the release of formaldehyde at the cell boundary (Holtzman, G.H. and Rossmore, H.W.; *Dev Ind Microbiol* 18:753, 1977). Short-term, high level exposure to respirable triazine aerosol/vapor would be expected to result in significant irritation, cellular damage and edema to the cells of the airways and alveoli.

Furthermore, the airborne triazine concentrations employed in this study do not have relevance to actual occupational exposure potential resulting from triazine use in metalworking fluids. Triazines are typically used at concentrations of less than 3% in metalworking fluids. A maximum theoretical triazine aerosol concentration of 0.15 mg/m³ would result if total oil mist levels were equal to the OSHA PEL of 5 mg/m³.

This maximum theoretical triazine concentration is 400 (analytical concentration) to 1500 (nominal concentration) times lower than the lowest exposure concentration employed in the mouse bioassay. In addition using the conservative assumption that triazine equals formaldehyde (on a mass equivalency basis), this level of triazine exposure (0.15 mg/m³) is below the threshold limit value (TLV) for formaldehyde (0.37 mg/m³).

For these reasons we do not consider the findings reported in this study to be indicative of a substantial risk pursuant to Section 8(e) of the Toxic Substances Control Act (TSCA). However, these studies are being submitted so that EPA has an opportunity to make its "own independent judgment", per its guidance. Please contact me about the final disposition of these studies. For future compliance purposes, we would like to know if EPA agrees with this analysis, or if EPA believes these studies are reportable, and if so why.

Sincerely,



Bradford H. Strohm, Ph.D.
Chemical Hazard Assessment & Toxicology

/ck

Enclosures

EVALUATION OF THE RESPIRATORY EFFECTS FROM COMPONENTS OF A
METALWORKING FLUID IN MICE

by

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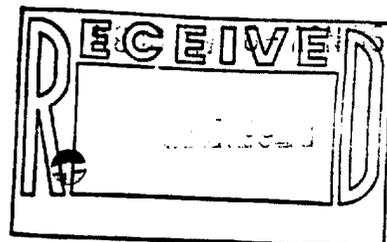
Submitted to the Graduate Faculty of
Environmental and Occupational Health,
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TABLE OF CONTENTS

I. Introduction.	1
A. Chemical Composition of Cutting Fluids.	6
B. Review of Epidemiology Studies	11
1. Chemical Carcinogenesis.	11
2. Skin Cancer.	14
3. Lung Cancer.	16
4. Gastrointestinal Tract Cancer.	18
5. Other Adverse Effects.	21
C. Review of Toxicology Studies.	24
D. Mouse Bioassay.	28
II. Methods.	32
A. Animals.	32
B. Machining Fluid Component Samples.	32
C. Generation of Test Exposures.	35
D. Exposure Chamber and Measurements of Animal Respiration.	36
E. Recognition of Irritation Responses.	37
F. Statistical Analysis.	39
III. Results.	40
A. Triazine.	45
B. Alkanolamides.	48
C. Boramide/Boric Acid.	51
D. Potassium soap.	52
E. Petroleum Oil.	52
F. Sodium Sulfonate.	53

IV.	Discussion.56
A.	Review of Component Actions.	56
B.	Determination of Sensory Irritants.61
C.	Determination of Pulmonary Irritants.62
D.	Cumulative and Sensitizing Effects.64
E.	Development of TLVs.66
F.	Threshold Limit Values for Mixtures.	68
G.	Determination of Concentration- Analytical versus Nominal.	69
H.	Recommendation of Exposure Limits.	70
V.	Appendix A Figures73
VI.	Appendix B Calculation of Nominal Concentrations. .	81
VII.	Bibliography.82

LIST OF TABLES

Table 1	General Composition of Cutting Fluids	10
Table 2	Components in Sample B	33
Table 3	Chemical Information on Components of Sample B.	34
Table 4	Summary of Qualitative Responses to Components of Sample B.	41
Table 5	Comparison of Exposure Concentrations Analytically and Nominally.	44
Table 6	Calculated Values for Components.	44
Table 7	Animal Mortality after Exposure to Triazine.	46
Table 8	Calculated Exposure Limits for Components.	67
Table 9	Calculation of the TLV Using ACGIH Mixture Formula.	69

LIST OF FIGURES

Figure 1	Sensory Irritation.	39
Figure 2	Pulmonary Irritation.	39
Figure 3	Concentration-Response Relationships for Sample B and its Components	43
Figure 4	Triazine exposures noting immediate decrease in f , rapid development of a plateau in response, and poor recovery following exposure.	46
Figure 5A	102 mg/m ³ Triazine exposure. Respiratory pattern seen at the beginning of exposure.	47
Figure 5B	Respiratory pattern seen 1 day after exposure	47
Figure 6	Alkanolamide #1, Variation in patterns of 2 mice exposed to 147 mg/m ³	49
Figure 7	Alkanolamide #1, 147 mg/m ³ , note large variation in f , particularly near the end of exposure	50
Figure 8	89 mg/m ³ Sodium sulfonate, 3 hour exposure.	55
Figure 9	80 mg/m ³ Sodium sulfonate, 4 hour exposure.	55

Introduction

It is estimated that more than 10 million U.S. workers are exposed to machining and grinding coolants and cutting oils (Jackson, 1992). This figure includes employees of many industries grouped together under the metalworking/machining classification, including those involved in drilling, grinding, cutting, textile production, mist lubrication and printing. Each application utilizes products designed to best meet the needs of the job, resulting in a diverse array of products available. Many modifications such as product dilution, are performed at the job site. These fluids have traditionally been composed of petroleum-based oils, often called "cutting fluids" or "cutting oils." New varieties of fluids are being produced, some of which are synthetic formulations that contain no petroleum oil.

Cutting fluid is used for two main purposes: for cooling, to prevent the distortion of the workpiece and to prolong tool life, and for lubrication to minimize frictional heat formation. The fluids are also useful in preventing rust formation and flushing away metal chips and swarf (Key et al., 1966).

Cutting fluids are generally sprayed in a continuous stream over the point of contact between the cutting edge of a machine tool and the metal being processed. At this point, there are minute quantities of the coolant being subjected to extremely high temperatures (approximately 800°C) and pressures due to friction, and cracking of the oil may occur

(Jepsen et al., 1977). Cutting oils and special lubricants which experience high temperatures or pressures may initially be aerosolized or volatilized. Some volatilized components will not recondense, resulting in worker exposure to vapors (Costa and Amdur, 1979). Condensation aerosols may also be produced. The oil spray is often collected under each machine and is usually filtered through a system of troughs before returning to a pump for recirculation. One lot of metalworking fluid will usually remain in use for several weeks or longer, with supplemental replenishment (Gilman and Vesselinovitch, 1955). Mists may also be produced by welding and heat treating of oily parts (Ayer, 1964).

In 1950, Cruickshank and Squire published the results of their research linking mineral oil exposure with an increased incidence of skin cancer in English industrial workers (Cruickshank and Squire, 1950). Since then, increasing interest has focused on the effects of machining fluids on the skin and organ systems. Potential adverse effects may be induced by cutaneous, inhalation and ingestion routes of exposure, after direct contact with fluids and with mists generated during machining operations. With the small aerosol that is formed (i.e., mass median aerodynamic diameter below 10 μm) significant deposition may occur in the respiratory tract (Ayer, 1964; Kennedy et al., 1989; Chan et al., 1990).

Particle size characteristics of workplace aerosols will vary with the process types and fluid distribution systems used. Lubricated machining operations involving impaction and spinning produce aerosols of relatively large size, while oil

mist lubrication systems produce much smaller particles with a median diameter of about $1.0 \mu\text{m}$ (Hendricks et al., 1962). Mists generated from synthetic and semi-synthetic fluids are often composed of smaller particles than those generated from straight or soluble oils (Quinn, 1992). As the diameter of airborne particles increases from $1\mu\text{m}$ to $10\mu\text{m}$, the deposition rate increases in the upper respiratory tract, where upward ciliary action tends to clear inhaled materials in the direction of the GI tract (Decoufle, 1978). This ciliary action will result in the ingestion of a significant proportion of oil mist particles, particularly when using straight oils.

Due to changes in products and manufacturing processes over the past 30 years, it is difficult to accurately assess worker exposure levels. Data presented by Hendricks et al. in 1962 suggested that average workplace exposure levels were below 15 mg/m^3 , but that higher levels were associated with certain jobs. For example, in the 1960s, it was not uncommon to find reports of automobile and steel manufacturing workplace concentrations of up to 56.5 mg/m^3 (Hendricks et al., 1962). However, in 1989, Kennedy et al. found total aerosol concentrations for machinists at two automotive factories ranging from 0.16 to 2.03 mg/m^3 . Modern usage of engineering controls, such as splash guards, and work practice controls that include good housekeeping and skin protection (i.e., arm guards, aprons, ointments) have also reduced exposure levels.

Both epidemiological analyses of occupationally exposed workers and animal models have been used to assess the potential hazards of oil mist exposure, and to determine safe exposure levels. Threshold Limit Values (TLV) are developed by the American Conference of Government Industrial Hygienists (ACGIH) as guidelines to the levels of airborne concentrations of substances under which it is believed that nearly all workers may be exposed repeatedly over the course of a working lifetime and suffer no adverse effects. "TLVs are based on the best available information from industrial experience, from experimental human and animal studies, and, when possible, from a combination of the three" (ACGIH, 1993). Many TLVs are based on the potential for sensory irritation. Humans exposed to sensory irritants may experience burning eyes, nose and throat, tearing, and coughing may occur (Alarie, 1973). The TLV-TWA (Threshold Limit Value-Time Weighted Average) (ACGIH, 1993) and the Permissible Exposure Limit (PEL) (U.S. Department of Labor, 1989) for an oil mist (mineral) are currently set at 5.0 mg/m³. Based on epidemiological studies of workers exposed to oil mists, adverse effects on humans have not been demonstrated at levels below 5 mg/m³. This level of exposure is also anticipated to minimize the potential for skin and respiratory tract irritation (ACGIH, 1991). It is felt that this TWA level may not be acceptable for all types of oils, due to potentially toxic additives which may be present (Lushbaugh et al., 1950). The documentation for this value (ACGIH, 1971) includes reviews by Hendricks et al.

(1962), and experimental studies by Lushbaugh et al. (1950), and Wagner et al. (1964). Lushbaugh et al. found no cancer or other significant changes in animals exposed to oil mist concentrations up to 132 mg/m³. Wagner et al. exposed five animal species to aerosol concentrations of 5 mg/m³ and 100 mg/m³. Their results suggested that while exposure to the higher concentration produced harmful effects in some animals, the 5 mg/m³ concentration presented no toxic hazard upon prolonged exposure.

The ACGIH has published a "Notice of Intended Changes for 1993-1994" which includes a TLV-TWA set at 0.2 mg/m³ for mildly treated oil products (ACGIH, 1993). This designation includes mildly solvent-refined, mildly hydrotreated, and mildly acid-treated oils, aromatic distillate extracts, catalytically treated oils and untreated oils. These products are also designated as category A1, confirmed human carcinogens, due to their polycyclic aromatic hydrocarbon (PAH) content. However, many of the newer types of machining fluids/cutting fluids do not contain oil, and it is not clear what their TLV should be. These products are currently considered as "Particulates Not Otherwise Classified" which have a TLV set at 5 mg/m³. There is no Short-Term Exposure Limit (STEL) recommended at this time, due to a lack of toxicological data and industrial hygiene experience on which to base this value (ACGIH, 1993).

The United Auto Workers (UAW) petitioned OSHA in December, 1993 for a tenfold reduction in the agency's current

permissible exposure limit of 5 mg/m³, citing the risk of cancer and other illnesses (Bureau of National Affairs, 1993). The union is also seeking the promulgation of an emergency OSHA standard and the development of an enforcement policy using the OSH Act's general duty clause to cite employers for exposing workers to harmful concentrations of oil mists. The UAW has petitioned the Environmental Protection Agency to promulgate a chemical test rule to obtain health effects studies on all machining fluids (Bureau of National Affairs, 1994). They have requested that chemical testing include mutagenicity studies, respiratory irritant and sensitization bioassays, chemical analysis of bulk products for degradation products, carcinogenicity bioassays, environmental monitoring, and human health effects studies.

Chemical Composition of Cutting Fluids

Mineral oils are mixtures of high molecular weight saturated hydrocarbons, derived from the controlled distillation of naturally occurring crude petroleum oils. Crude oils are first distilled at normal atmospheric pressure, then under high vacuum yielding vacuum distillates and residual fractions that can be further refined to mineral base oils (Spivey, 1988). The source of the original crude oil and the processing used will determine the final chemical composition of the mineral base oil. Lubricant-based oils are complex mixtures of straight and branched chain paraffinic, naphthenic (cycloparaffinic) and aromatic hydrocarbons.

The various types of oils have been categorized into the following classes (Bingham, 1980):

Class 1 Vacuum distillates- These may have undergone subsequent finishing steps, such as neutralization with alkali, dewaxing, clay treatment, and/or mild hydrotreatment but have not been acid treated or solvent extracted.

Class 2 Acid-treated oils- These may have undergone subsequent finishing steps, such as neutralization with alkali, dewaxing, clay treatment and/or mild hydrotreatment. They have not been solvent extracted.

Class 3 Solvent refined oils (raffinates)- These may have undergone subsequent treatment steps, such as dewaxing, clay treatment and/or mild hydrotreatment.

Class 4 Hydrotreated oils

Class 5 White oils and petroleums suitable for food or medicinal use- These are highly refined, colorless oils free of all unsaturated compounds, aromatic compounds and other constituents that influence color, odor and taste.

Medicinal grade, or laxative mineral oil is composed mainly of naphthenic hydrocarbons. The U.S. Food and Drug Administration permits the use of odorless light petroleum hydrocarbons (boiling point $<343^{\circ}$ C) as direct or indirect food additives (Bingham, et al., 1980). Paraffin oil is a blend of straight and branched aliphatic hydrocarbons. Light lubricating oil was designed for use in the textile industry,

achieving its lubricating properties without the use of additives. It is composed approximately of 2 parts paraffinic oil and 1 part naphthenic oil, plus approximately 1% aromatic hydrocarbons (Costa, 1979).

Several broad categories of machining fluids have been used in industry (see Table 1). Cutting oils are based on highly refined oils (Classes 3 & 4) and oils derived from limited refining. The oldest types used are petroleum based mineral oils known as "straight cutting oils." Due to their high lubricating qualities, these products are still widely used. Emulsions of mineral oils in water are known as "soluble oils." These products have been used extensively since the 1940s. While the oil component provides reasonable lubrication, the water component has excellent cooling properties. Transparent emulsified oils contain more detergent, and are often known as semisynthetic fluids (Jarvholm, 1981). More recently, machining fluids have been developed that use synthetic chemicals only, with no mineral oils. These "synthetic" fluids are water soluble and are used primarily for their cooling properties, but they also have acceptable lubricating qualities (Kennedy et al., 1989). These solutions are generally alkaline (approximately pH 9) and are frequently hypoosmolar. There is considerable evidence that the higher pH associated with synthetic and semisynthetic products may increase the potential for their aerosols to cause respiratory complaints (Gordon, 1992). While traditionally, straight cutting oils have been used for most applications, they are

slowly being replaced by synthetics and soluble oils due to cost advantages and industrial waste disposal requirements. Soluble oil and synthetic coolants are sterile when manufactured, however after being mixed with water and used, a variety of bacteria and fungi may grow, including endotoxin producing gram negative bacteria (Key et al., 1966).

Ronneberg and Skyberg (1988) noted that the toxicological properties of mineral oil products will vary with refining and distillation histories, derivation of the crude petroleum, and the types and amounts of additives present in the final product. Industrial oils used for cutting, drilling or grinding may contain many additives, including amines, which act as emulsifiers, and nitrites, which act as corrosion inhibitors (Bingham, et al., 1980). The presence of additives, and their percentage in the product in use will vary between operations. Often these variables will be determined at the plant level, and therefore the composition of the fluids may differ from plant to plant (Eisen et al., 1992). Varying amounts of contaminants may be present, and may include compounds containing a large number of hydrocarbons (Ayer, 1964).

Table 1 General Composition of Cutting Fluids

<p><u>Straight Oil:</u> mineral oil base(naphthenic or paraffinic) 60-100% extreme pressure additives*: sulfur 1-3% chlorinated fats & paraffin oils 1-3% sulfochlorinated fats 1-3%</p>
<p><u>Soluble Oil(emulsifiable):</u> mineral oil-30-85% (v/v) emulsifiers (e.g. sulphonates, soaps, ethoxylated alcohols) biocides* corrosion inhibitors* (e.g. hydroxyl amines, nitrites) extreme pressure additives* antifoaming agents* (e.g. silicones) dyes* water conditioners* (e.g. polyphosphates, borax, sodium carbonate) Water-added to concentrate prior to usage</p>
<p><u>Semi-synthetic/Synthetic Coolant:</u> Oils (5-30% in semi-synthetics only) corrosion inhibitors soaps and wetting agents* blending agents* (e.g. glycols) biocides* water conditioners* antifoaming agents* dyes* Water- plus additional dilution performed at workplace</p>

* additives not present in all formulations
(Key et al., 1966)

Review of Epidemiology Studies

Chemical Carcinogenesis

Cutting fluid formulations have been associated with increased incidence of cancer, with various conclusions reached in epidemiological studies. Different formulations of oils have been associated with specific body site cancers. Exposure to straight oils has been linked to increased incidence of rectal, laryngeal and prostatic cancers. Tolbert et al. (1992) found the standardized mortality ratio (SMR) for workers exposed to straight oils was elevated, with some indication in the Poisson modeling that risk increased with increasing duration of exposure. Jackson's findings (1992) suggested a positive association of rectal cancer with straight oil, stomach cancer with soluble oil and grinding, laryngeal cancer with straight and soluble oils, prostatic cancer with soluble oil, synthetic machining fluids and grinding, brain and pancreatic cancer with soluble oils, and leukemia and lung cancer with all exposure groups.

There is evidence that additives commonly used to improve the performance of an oil product may form carcinogenic compounds and that the processing of a product may also influence its carcinogenicity. In 1987, Jarvholm and Lavenius noted that N-phenyl-2-naphthylamine, a highly suspected bladder carcinogen, has been listed as a constituent in cutting fluids in the Federal Republic of Germany. Lower cancer incidence rates found in their investigations of oil exposed Swedish workers were partially attributed to the use

of Swedish products which did not contain known carcinogens. Workers with longer histories of occupational exposure to cutting oils often will have worked with a variety of machining fluids, which makes an epidemiological follow up more involved.

There is some epidemiological evidence to suggest that N-nitrosamines or other nitroso compounds may be related to increased incidence of cancer in certain areas of the world. Nitrosamines are mutagenic and N-nitrosodimethylamine has been listed as a suspected human carcinogen (ACGIH, 1993). Humans poisoned by nitrosamines show acute, subacute and chronic pathological changes; human cells and organs in culture may undergo malignant transformation after exposure to N-nitrosamines (Wagner et al., 1964). In 1976, the National Institute for Occupational Safety and Health (NIOSH) stated in a Current Intelligence Bulletin that potentially carcinogenic nitrosamines may be found in synthetic, semisynthetic and soluble cutting oils. During short periods of time from 1961 onward, dialkanolamines and sodium nitrite have been used simultaneously in cutting fluid formulations, resulting in increased potential for nitrosamine formation (Jarvholm et al., 1981). While nitrosamines may be found in the workplace as contaminants, there has been no evidence to suggest that occupational exposure has influenced cancer incidence rates (Spivey, 1988).

Straight cutting oils have not been identified as containing nitrosamines, but these water insoluble oils may

contain polycyclic aromatic hydrocarbons (PAHs), which are recognized as potential carcinogens (Bingham, 1980). Benzo(a)pyrene (BaP) has been used as an indicator compound for the estimation of carcinogenic potential of complex, PAH-containing materials. Bingham (1980) identified BaP in most, but not all unused petroleum-derived oils, with concentrations varying between 0 and $170\mu\text{g/l}$. Used oils were found to contain higher concentrations, up to $250\mu\text{g/l}$.

In cutting oils used at temperatures higher than 800°C , formation of PAH occurs, with concentrations of benzo(a)pyrene increasing up to 100 fold (Granello and Clonfero, 1991). Apostoli et al. (1993) evaluated the time-related variation in concentrations of PAHs in cutting fluids, finding that although samples obtained after nine months of use had the highest PAH levels, the maximum increase in PAH concentration occurs between the third and sixth month of use.

The proportions and types of PAHs in finished oils are refining process dependent. Mild processing, such as acid-clay, reduces the total aromatic content of an oil slightly, but does not reduce the concentration of PAH. Mild hydroprocessing reduces the PAH content, but has little effect on the aromatic hydrocarbon content. Solvent extraction, or severe hydroprocessing reduces both the PAH and aromatic hydrocarbon content substantially. Severe treatment with oleum can remove almost all aromatic hydrocarbons, including PAHs, to yield the medicinal grade products (Bingham, 1980). Most mineral oils used today are solvent-refined, with much

less PAH present, however, acid-refined mineral oils may still contain sufficient quantities of PAH to cause skin cancer in animal models (Jarvholm and Lavenius, 1987).

Granello and Clonfero (1991) suggested that occupational exposure to PAHs and mutagens in the metal industry can be reduced by using solvent refined oils at low temperatures and by periodically changing the oils. Apostoli et al. (1993) further suggested that cutting fluids be changed at least every six months.

Skin Cancer

Since the latter half of the nineteenth century, there have been reports describing carcinogenic effects for the skin associated with intense and prolonged exposure to various types of mineral oil (Decoufle, 1976). Initial observations from the British Isles and Europe noted that workers engaged in various occupations including shale oil, coal oil and petroleum refining, cotton mule spinners, and machine tool operators were developing "paraffin cancers" (Decoufle, 1976). While exposure to cutting oils was well recognized as a cause of dermatitis, a strong association to cancer was not demonstrated until 1950.

Cruickshank and Squire (1950) investigated cases of scrotal cancer that occurred in workers employed in the light engineering industries of the English Midlands between 1939 and 1948. The British cotton spinning industry workers known as "mule spinners" were often heavily exposed to the

lubricating oils used on the machine spindles. While these men had an increased incidence of scrotal and other skin cancers, men who operated wool mules, which operated at slower spindle speeds, had almost no cancer incidence. Increased worker exposure to mineral oils due to splashing and aerosolization from the faster moving spindles was suggested as the cause of the increased cancer incidence. Cruickshank and Squire then utilized biologic tests on animals to verify their clinical observations that exposure to cutting oils was associated with squamous-cell carcinoma. Such reports of epitheliomas of the hands, arms and scrotums of machine tool operators in England prompted the institution of a mandatory notification system for cancer, to identify potentially work-related disease. The increased risk of scrotal cancer is now believed to be more an effect of splashing than of oil mist inhalation (Jarvholm et al., 1981).

The mode of action of chemical carcinogens on skin has been studied extensively. Fibroblasts cultured from oil-affected skin appeared to show a higher frequency of chromosomally abnormal subpopulations than those obtained from unexposed skin areas (Mackerer, 1989). Spivey noted in 1988 that only mildly treated solvent refined oils produced skin tumors, while severely treated oils did not. A combination of solvent extraction and hydrotreatment reduced or eliminated skin tumorigenicity (Spivey, 1988). It appears that at least 15 years of exposure to the oil products may be necessary for premalignant lesions and keratoacanthomas to develop (Jarvholm

et al., 1985). Oil exposed workers are often aware of their increased skin cancer risks. By seeking medical care promptly, some lesions may be treated in the premalignant stage. Morbidity studies based on cancer registrations therefore underestimate the risk to workers (Jarvholm et al., 1985).

Lung Cancer

The effects of inhalation of mineral oil aerosols have been studied extensively, with results obtained from various investigations often leading to different conclusions. Most oils will evoke reactions when they gain sufficient access to the bronchial and pulmonary spaces. The type and severity of reactions varies with the fluid type used and the presence of any additives in the product. The relationship of oil mist inhalation to pulmonary cancer is still uncertain. Prolonged, repeated exposure to significant atmospheric levels of mineral oil has been shown to cause bronchopulmonary symptoms, followed by lung fibrosis. However, epidemiological evidence of malignant or nonmalignant tumorigenesis is limited. Determination of causal relationships between occupational exposure to mineral oil mists and lung cancer is further complicated by an estimated latency period of 20 years or more before tumor development (Ronnenberg et al., 1988). The lack of correlation between known carcinogenic potential for skin and the ability to produce lung tumors also makes these investigations more difficult (Gilman and Vesselinovich, 1955). Decoufle (1978) found no unexpected mortality from

respiratory cancer among men engaged in metal machining jobs, but Ronneberg et al. (1988) concluded that mineral oil exposure was probably an important contributing factor in the development of lung cancer in Norwegian cable manufacturing workers, finding nearly a three-fold increase in lung cancer incidence among these workers. Jarvholm and Lavenius (1987) found no increased mortality, incidence of lung cancer or death resulting from nonmalignant respiratory disease when comparing a cohort of 792 men occupationally exposed to mineral oils and cutting fluids to the general population of the city. Among the cohort of Swedish workers exposed to oil mist for five years or more, only three cases of lung cancer were identified, compared to an expected 5.4 cases. Men employed as grinders had nearly twice as many cases of stomach cancer as expected, however due to the small numbers, this was not considered statistically significant. Comparing 126 total worker deaths with the 154.3 expected deaths calculated from population mortality rates may give a false sense of confidence, due to what is termed the "healthy worker effect" (Jarvholm, 1981). When comparing workers with general population figures, which include persons with serious medical conditions who are unable to work, worker morbidity and mortality rates will appear artificially low since the healthier members of the community are included in the worker data.

Negative findings were obtained in lung cancer investigations by several researchers. Tolbert et al. (1992)

found a stable inverse dose-response trend in their analysis of workers exposed to water-based fluids (synthetic and soluble), while there was no association with straight oil exposure.

Gastrointestinal Tract Cancer

If the inhalation of oil mists may result in ingestion, the gastrointestinal system must also be considered as a potentially affected site. Cancers in the GI tract are relatively common in the general population, therefore it is more difficult to identify increased incidence in those occupationally exposed to oil mists.

Workers exposed to petroleum products have increased incidence of stomach, colon and rectal cancers, but their mortality rates have not been striking or statistically significant (Bingham, 1980). Reviewing a subgroup of workers exposed to cutting oils for more than five years prior to 1938, Decoufle (1978) found that their combined risk of stomach and large intestine cancers was double the expected rate. The twenty year follow up period required to identify this increased GI tract cancer incidence highlights the possibility that an extended latency period may be associated with oil mist exposure. It is very difficult to determine dose/response data since exposure concentrations have not been consistently monitored or documented over such extensive time periods. Tolbert et al. (1992) identified a significant association of rectal cancer incidence with years of exposure

to straight oils. Workers with greater than 7.5 years of exposure were found to have a relative risk of 3.2 ($p < 0.001$).

Wang et al. (1983) noted excess cases of gastrointestinal cancers in workers involved in glass lens production. Workers hands were noted to be constantly and heavily covered with materials from grinding operations, leading to the hypothesis that they may have also ingested abrasives and pitch through contaminated food and drink.

Chemical carcinogenesis has been suggested as a potential cause of the increasing incidence of pancreatic cancer in the United States. A ten year mortality study by Vena et al. (1985) found a particularly high proportional mortality ratio (PMR) of 1.9 for white autoworkers developing pancreatic cancer. Evaluating white machinists in the Los Angeles area, Mack and Paganini-Hill (1981) reported a PMR of 1.3 for pancreatic cancer. While ethnicity is believed to influence cancer risks, with foreign born white males having a higher cancer incidence rate than those men who were born in the U.S., this trend is especially evident involving GI tract cancers (Haenzel, 1961; Mack and Paganini-Hill, 1981). However, most epidemiological studies have not stratified their white male groups into native versus foreign-born. Any further evaluation of the influence of native origin on cancer data could only be estimated from local population demographics.

One other variable must be considered in any epidemiological investigations of cancer incidence. It is believed that individuals may vary in their susceptibility to cancer.

Jarvholm et al. (1985) did not consider exposure differences to be a satisfactory explanation for the increased incidence of skin lesions found in some individuals. Differences in the ability to induce aryl hydrocarbon hydrolase and variability in man's ability to repair DNA have been proposed as explanations for individual susceptibility variations (Jarvholm et al., 1985). Increased susceptibility to cancer may also be genetically predetermined or acquired during or after carcinogen exposure. Due to individual variations in susceptibility to cancer, Jarvholm et al. (1985) recommended that workers who have a malignant or premalignant skin lesion or keratoacanthoma change to a job that doesn't require exposure to oil.

Other Adverse Effects

Respiratory Tract Irritation

Workers exposed to oil mists may also display symptoms of respiratory tract irritation (e.g., chronic cough, chronic phlegm production). Although exposures to various types of cutting fluids with different particle sizes have been evaluated, worker complaints have indicated widespread discomfort at concentrations greater than 5 mg/m³ (Tolbert et al., 1992). Jarvholm (1982) reviewed changes in the respiratory symptoms of workers for 3 years after they experienced complaints (e.g., chronic bronchitis, dyspnea) associated with workplace exposure to oil mists. Changes in symptoms or progression of respiratory difficulty did not differ from non-exposed control workers.

Kennedy et al. (1989) evaluated workers before and after the work shift to determine if oil mist exposure caused any acute respiratory effects. Spirometry was performed on four occasions for each subject: on a Monday, before and after the work shift, and similarly on Friday. While a measurable decrease in forced expiratory volume in 1 second (FEV₁) was seen after the completion of the work shift, no progressive decline was noted during the week. Results were adjusted statistically for history of childhood asthma, smoking prior to pulmonary function testing and for race. Odds ratios for a 5% or greater decrease in FEV₁ response were 4.4 among workers exposed to aerosols of straight mineral oils, 5.8 for oil emulsions and 6.9 for synthetic fluids. Cross shift decreases were associated with inhalable aerosol levels greater than 0.20 mg/m³, indicating that acute airway response may occur well below the current recommended exposure limits. While FEV₁ decreased with higher exposure levels, these changes appeared completely reversible. Mild airway response is common with straight oil and emulsion exposures, but whether this may indicate a nonspecific "irritant" airway effect or airway sensitization to an oil mist component has not been determined.

Oxhoj et al. (1982) compared respiratory symptoms of oil exposed workers to those of a general population of Swedish and Danish men. While no spirometric differences were found between these groups, a significant positive relationship was shown relating the prevalence of cough and phlegm production

with the concentration of oil aerosol present in the work environment of smokers. The concentration of oil vapor was not related to any symptoms, suggesting that the causative agent was not the oil itself, but something in or on the oil droplets.

Dalbey et al. (1981) expressed concern that workers with respiratory symptoms associated with oil mist exposure that did not have abnormal chest xrays would not be considered to be adversely affected, leading to serious underestimation of the toxicity of oil mists. They hypothesized that workers affected by oil mist exposure might leave that type of work environment before radiographic or functional changes would occur, due to work-associated respiratory discomfort.

Oil aerosols have been shown to alter the effects of other irritants that might be present in the workplace. Exposure to mineral oil mists may enhance sensory irritation caused by exposure to chemicals such as formaldehyde, chloroform, acrolein or tincture of capsicum (Magill, 1976). Costa and Amdur (1979) found that paraffinic mineral oil aerosols lessen the irritant response produced by sulfur dioxide. It is interesting to note that Drasch et al. (1974) identified that oil mist exposed employees of various German metal working companies had been reported to have had decreased respiratory complaints associated with smoking. They also speculated that chronic exposures to oil mists at concentrations of 40-150 mg/m³ could actually protect workers from the effects of smoking.

There is considerable evidence that the pH or titrable acidity can influence the potential for aerosols to cause acute respiratory effects. Several studies have demonstrated that airway dysfunction occurs after exposure to aerosols with a pH significantly different from the normal 6.6 value of the fluid layer of the airways (Koenig et al., 1983; Gordon, 1992).

Noncancerous Skin Effects

Oil acne, folliculitis, chloracne, irritant contact dermatitis and allergic contact dermatitis have been documented after skin exposure to oil mist (Hendricks et al., 1962). Bacterial contamination of lubricant coolants may contribute to the breakdown of the product, but this microbial growth has not been associated with outbreaks of folliculitis (Hendricks et al., 1962). While dermatitis remains the most prevalent health effect seen after exposure to oil aerosols (Mackerer, 1989), improved personal hygiene for workers has done much to reduce the incidence of this problem.

Lipid Pneumonia

One of the earliest concerns of oil aerosol exposure was the occurrence of lipid pneumonia. This pulmonary problem may result whenever an exogenous lipid reaches the lungs and remains there for a sufficient length of time, causing irritation (Lushbaugh et al., 1950). Although lipid pneumonia is rare, most of the limited number of reported cases have

resulted from the ingestion or aspiration of oils used for medicinal purposes. This type of pneumonia can be a serious, debilitating disease.

Review of Toxicology Studies

To date, direct evidence of carcinogenic effects in animals from inhalation of mineral oils has not been found. At relatively low levels of exposure, mice, rats and rabbits have not developed pulmonary lesions. There appears to be a great deal of variability in respiratory tract function between animal species. For example, after exposure to oil mist concentrations which produced no effects in other animal species, monkeys have shown a greatly increased incidence of focal pneumonia, interstitial inflammation and acute gastritis (Lushbaugh et al., 1950; Shoshkes et al., 1950; Wagner et al., 1964). Lushbaugh et al. (1950) exposed animals to aerosolized automobile lubricating oil and diesel engine lubricating oil at concentrations ranging from 63 to 132 mg/m³. Oil did not accumulate in the alveoli at a fast enough rate to overcome the ability of the pulmonary phagocytes to engulf and remove it when the lower concentrations of oil were used.

Since only the smallest droplets (<3.5 μm) are considered to be inhalable (ACGIH, 1993), single macrophages are capable of removing many of the oil droplets. The larger particles that enter the alveoli tend to be retained, while those smaller than 1 μm in diameter are exhaled in considerable percentages. The larger portion of the total mass of

particles retained in all divisions of the respiratory tree would be supplied by the small number of larger size particles that impacted in the lung divisions, delivering much more mass than numerous smaller particles (Shoshkes et al., 1950). Shoshkes et al. (1950) found that in mice exposed to mists with an average particle mass median diameter of 2.5 μm , droplets were evenly dispersed throughout the lung. In each lung, the highest concentrations of particles were in and around the terminal bronchioles and alveolar ducts. Since these areas contain the most surface area as well as the narrowest lumens of the lung airway, they provide the largest surface area for oil mist droplet impaction. The phagocytosis of oil droplets was essentially complete within 48 hours after aspiration of oil mist, except after prolonged exposures, where large numbers of free droplets could be seen in the lungs. Except for the appearance of scattered macrophages, no acute inflammatory changes were seen in the lungs after a 2 hour exposure. After prolonged heavy exposures, for periods extending from 2 to 4 weeks, liquid petrolatum and motor oil droplet retention resulted in localized foreign body reactions of moderate severity. Comparison of mouse and human lung sections revealed only a slight discrepancy between the dimension of the alveoli and the terminal bronchioles. Shoshkes et al. (1950) found that particles retained in mouse alveoli had a mass median diameter of 0.8 μm , which correlated well with human studies performed by Wilson and LaMer (1948) showing maximum alveolar retention of particles ranged from 0.6 to 2.0

μm . Therefore, histopathology information obtained from mouse exposures was assumed to closely predict responses in humans.

Dalbey et al. (1991) exposed Sprague-Dawley rats to oil aerosols generated from three base stocks, each produced by a different method of refining. Treatment-related effects were only seen in the lung and tracheobronchial lymph nodes. Foamy macrophages with numerous vacuoles of varying size were present in the alveolar spaces of many of the exposed animals, and were dose-related in number. The foamy macrophages were most prevalent in the alveoli close to the alveolar ducts. In contrast, Yevich (1965) found that although dogs exposed to the mineral oils had oil-filled macrophages seen in the alveoli, the main effects of exposure were seen at the respiratory bronchiole. After twenty-six months of exposure, free oil droplets had accumulated in the bronchiolar walls, causing the complete destruction of the walls. The lumen of some respiratory bronchioles were obliterated by lipid granulomas.

Skyberg et al. (1990) exposed male Wistar rats to two mineral oils and three synthetic fluids to compare oil products in use in the cable manufacturing industry with alternative products under consideration. Not all vacuoles in the alveolar macrophages contained oil and polymorphonuclear cells were not prominent. This vacuolization pattern was felt to represent a cytotoxic effect and be a precursor to pulmonary fibrosis.

Stula and Kwon (1978) found that rats exposed to oil aerosol developed oil granulomas, alveolar epithelial cuboidal changes and/or bronchiolar proliferation, along with cholesterol-like clefts. Attempts to expose rats to 1.5 mg/l (1500 mg/m³) of oil aerosol for 5 hours per day by Selgrade et al. (1990) resulted in 60% animal mortality after only 2 days of exposure, apparently due to pulmonary hemorrhage. Microgranulomas were still present in animals sacrificed after a 10 month recovery period. Although it has been hypothesized that these lesions may remain for the entire lifetime of the rat, research using mice and gerbils indicates that oil clearance systems found in their lungs are more efficient.

Biocides may be added to a product to prevent the growth of microorganisms. Microbial contamination of machining fluids not only leads to product breakdown, but endotoxin producing gram negative bacteria and fungi may proliferate (Rossmore, 1981). Many biocides release formaldehyde, which has been shown to concentrate and produce tumors in the nares of mice and rats (Mackerer, 1989), and is classified as a suspected human carcinogen (A2) by the ACGIH. Earlier risk assessments on formaldehyde exposure are flawed, as new information concerning its mechanism of action have shown that the dose of formaldehyde administered is not linearly proportional to the dose delivered to the target tissues (Mackerer, 1989). Organic sulfur compounds are widely used as additives in cutting oils. The role of sulfur and sulfur compounds in tumorigenesis has also been reviewed (Bingham, 1980).

Since about 1950, solvent dewaxing has been used to process crude wax. The entire oily distillate is first dissolved into a solvent such as methylethylketone, then after a chilling process, the wax can be filtered from the oil-solvent mixture (Bingham, 1980). It was noted by Smith et al. in 1951, and Hendricks in 1959 that the aromatic extracts separated from crude wax were more carcinogenic to animals than the crude wax before processing.

Mouse Bioassay

In 1966, Alarie described an animal bioassay using Swiss-Webster mice which could assess the degree of sensory irritation caused by airborne chemicals. Humans exposed to sensory irritants may experience burning eyes, nose and throat, tearing, and coughing may occur. These reflex reactions are due to chemical stimulation of the trigeminal nerve endings in the nasal mucosa or the laryngeal nerve endings in the throat. Irritation of the lower airways is known as pulmonary irritation and is due to stimulation of the vagal nerve endings. Pulmonary irritants produce a sensation of dyspnea and breathlessness in humans and may produce pulmonary edema, possibly resulting in death (Alarie, 1966; Alarie, 1973). The conducting airways of the lungs may be constricted by airborne chemicals known as bronchoconstrictors or airway constrictors. All three types of reactions may result in reduced breathing frequency. With sensory irritants, this is probably an involuntary response to protect the lungs from further insult.

It would be difficult and potentially dangerous to expose humans to various compounds to test for potential irritancy. The mouse bioassay is a rapid, inexpensive toxicological evaluation which may be used to predict the type(s) of adverse reactions which humans may experience when exposed to various chemicals (Alarie, 1966).

The mouse bioassay evaluates the changes in respiratory pattern and frequency (f) during an exposure to an airborne chemical. It is an American Society for Testing and Materials (ASTM) (1984) standard method for estimating sensory irritancy of airborne chemicals. Mice are exposed to different concentrations of an airborne chemical, so that a concentration-response relationship may be determined. As decreases in f may result from different reactions, sensory irritation must be verified through inspection of respiratory patterns. The concentration-response curve is obtained by plotting the percent decreases in f from control conditions against the logarithm of the concentration of the airborne chemical (Alarie, 1966). The concentration inducing a 50% decrease in f , known as the RD_{50} , may then be determined. Relative comparisons of potency may be made using the RD_{50} values.

Alarie has shown that the mouse bioassay is predictive of human responses (Alarie, 1973). Humans recognize sensory irritants with eye, nose and throat sensations when mice exhibit a characteristic change in their breathing pattern (i.e., "braking" during expiration). To "calibrate" the mouse bioassay to human responses, Alarie (1973), Kane et al. (1979) and

Alarie et al. (1980) showed that humans exposed to a chemical at its RD_{50} would experience intolerable burning of the eyes, nose and throat. They also predicted that slight irritation would occur at $0.1 \times RD_{50}$ and at $0.01 \times RD_{50}$, minimal or no effect would occur. Acceptable levels for human exposure would be expected to fall within this range, thus 0.03, the midpoint between 0.1 and 0.01 on a logarithmic scale was selected as an appropriate factor for multiplication of the RD_{50} , to establish safe levels for human exposure in developing TLVs for sensory irritants (Alarie, 1981; Alarie and Luo, 1986).

Schaper (1993) recently developed a database containing 295 airborne materials which had been evaluated for sensory irritating properties using the mouse bioassay. Using the analytic approach previously used by Alarie (1981) and by Alarie and Luo (1986) to compare TLVs and RD_{50} values, a strong relationship was confirmed between the TLV and $0.03 \times RD_{50}$.

Schaper and Detwiler (1991) evaluated the acute respiratory effects produced by 10 machining fluids using the mouse bioassay. They identified significant differences in biological potency between the samples tested. A semisynthetic product, identified as "Neat Sample B," produced sensory and pulmonary irritation, and was the second most potent fluid tested with an RD_{50} of 154 mg/m^3 . The manufacturer has indicated that the final formulation of this product includes seven components, plus water. The present study was undertaken to evaluate the components of this fluid, in an attempt to

identify the compound(s) responsible for the acute respiratory effects seen by Schaper and Detwiler. It has been suggested that the current PEL of 5 mg/m³ may need to be lowered for this product to prevent worker irritation (Schaper and Detwiler, 1991; Kennedy et al., 1989). If the more potent components identified in this study could be modified or eliminated in the final product, a less irritating fluid could be developed.

Methods

Animals

Specific pathogen-free, male, Swiss-Webster mice were obtained from Hilltop Lab Animals (Scottsdale, PA). They were housed 4 to a cage in an animal room on a 12-hr. dark/light cycle. Food (Purina Chow) and water were provided *ad libitum*, except during exposures. A new group of four mice, weighing 24-28 g was used for each experiment.

Machining Fluid Component Samples

Eight components of Sample B were obtained from the manufacturer. During production, boric acid is added to the mixture and is expected to react with amines present to produce boramide. Both boric acid and boramide were provided for testing purposes (see Tables 2 and 3). Fluids were stored in original sealed containers at room temperature. After opening a container, the product was kept in a cold room (approximately 5° C) until it was needed again. Characteristics of each component were noted, to verify that no change from the original state had occurred. Due to component viscosity, or granular form (Boric acid), certain samples had to be diluted with distilled, deionized water to produce a test solution.

Table 2 Components in Sample B

Component	Per- cent of com- pound	Physical description	Use conc. for ex- posure	Product function
Alkanolamide #1	5%	dark brown, thick liquid	neat*	Emulsifier, Extreme pressure additive
Alkanolamide #2	5%	dark amber, thick liquid	neat	Emulsifier, Extreme pressure additive
Boramide	5%	pale yellow, oily liquid, very tacky	50% w/v	Coupling agent
Boric acid	**	white, crystalline powder	<10% w/v, saturated solution	Coupling agent, picks up amines
Petroleum Oil	20%	pale yellow oily liquid	neat	Lubricant
Sodium Sul- fonate	3%	brown, very viscous, semisolid	7% w/v	Emulsifier
Triazine	3%	amber liquid thicker than corn oil	neat	Biocide, Rust inhibitor
Potassium Soap	5%	dark amber liquid, moderately viscous	10% w/v	Emulsifier
Water	54%			Diluent, coolant

* "Neat" indicates that the component is not diluted

**Manufacturer introduces *boric acid* to the product, but expects *boramide* to be formed.

Table 3 Chemical Information on Components of Sample B

Component	pH	CAS #	Chemical Description	VP mm Hg
Alkanolamide #1	10.2	68155-20-4	amides, tall oil, fatty, N,N-bis(hydroxyethyl)	N/A
Boramide	9.5	68516-78-9	boric acid compound + 2,2',2"-nitrilotris(ethanol)	N/A
Boric acid	5.1	10043-35-3	BH ₃ O ₃	15
Petroleum Oil	5.8	64742-52-5	hydrotreated heavy naphthenic petroleum distillates	0.1
Sodium Sulfonate	10.0	68608-26-4	Sulfonic acids, petroleum, sodium salts	<1
Alkanolamide #2	9.4	3077-30-3	Octanamide, N,N,-bis(2-hydroxyethyl)-	N/A
Triazine	9.1	4719-04-4	1,3,5-Triazine-1,3,5(2H,4H,6H)-triethanol	15
Potassium Soap	9.8	68649-05-8	Butanoic acid, 3-amino,N-coco alkyl derivative	N/A

Generation of Text Exposures

Using a Harvard Apparatus syringe pump, each component solution was fed into a Pitt No. 1 (Wong and Alarie, 1982) or a Pitt No. 4 (Rosato et al., 1988) aerosol generator. The Pitt No. 4 generator was used to generate higher concentrations of aerosols for certain exposures. The rate of fluid delivery to the generator varied from approximately 0.015 to 3.3 ml/min. Dried, compressed air was delivered to the generator, (Pitt #1, 12 psi, Pitt #4, 20 psi) producing a mist as the fluid met the air stream at the jet of the generator. All mists were examined with a light source to assure that a fine mist was indeed being produced. Generator output was approximately 10 liters of air/min., which was directed into the mouse exposure chamber (described below). Mist concentration was determined at least five times during each exposure. Air samples were drawn at a rate of 2 l/min from the mouse exposure chamber onto Gelman, type A/E glass fiber filters (47 mm diameter, Gelman Sciences, Inc., Ann Arbor, MI). All filters were weighed using a Mettler balance (Model AE240, Mettler Instrument Corp., Hightstown, NJ). A Marple personal cascade impactor (Model 290, Andersen Samplers, Inc., Atlanta, GA) was used for sizing the aerosols.

The gravimetric analysis of aerosolized components reflects only the solid content and low vapor pressure components of each fluid. Although many of the components were diluted significantly with water, the large volumes of dried air used for aerosolization and chamber exhaust would have

permitted the vaporization of the water phase. High vapor pressure components present in the fluids would not have been retained on the filters used. Exposure concentrations listed in the Results section therefore only reflect non-volatilized or solid components, with no additional water included.

Exposure Chamber and Measurements of Animal Respiration

The exposure chamber was made of glass, with a volume of 2.5 liters. Each chamber had four body plethysmographs attached to it, as described by Barrow et al. (1977). Four naive mice were used for each exposure, each positioned in a body plethysmograph. The head of each mouse extended through a 6 mm dental latex dam positioned within an 8 mm tape collar, which was securely taped to the inside of the chamber. The heads of the four mice were thus held by a comfortable, airtight seal in the interior of the exposure chamber. Rubber stoppers sealed the end of each plethysmograph and prevented the mice from escaping. A flowmeter was used to monitor the exhaust airflow through the chamber, with continuous ventilation drawn at a rate of 20 liter/min. A sensitive pressure transducer was attached to each body plethysmograph (Gaeltec 8T-2, Hackensack, NJ) to measure the plethysmographic pressure changes created by the inhalation and exhalation of the mice. Transducer output was directed into a Gould RS 3400 4-channel recorder. Each channel continuously monitored the respiration pressure changes of an individual mouse. All signals (analog) were digitized at a

rate of 200 samples/sec. using a Metrabyte analog to digital converter (Model DAS-16) and stored on a Trillian Power Systems personal computer (Model II, 386 chip).

The amplitude of the plethysmographic pressure changes, corresponding to thoracic displacement, was taken as tidal volume (VT). Calibration of VT was not performed, as only relative changes in VT were of interest here. Breathing frequencies (f) were measured for each of the four mice by counting the number of pressure waves per unit time. Frequencies were recorded every 15 seconds and displayed on the computer monitor. Following exposure, VT and f were plotted as a function of time for each mouse. Mean tidal volume and mean respiratory frequency (± 1 standard deviation) were plotted as a function of time.

Each experiment consisted of a 20 minute control period to establish a baseline frequency, followed by a 180 minute exposure period. The animals were monitored for an additional 20 minutes after aerosol generation had ended to assess recovery. After the animals were removed from the exposure chamber, they were visually observed to detect any unusual behavior patterns or physical characteristics. Mice were visually checked for several days. One or more groups from each component were monitored by plethysmography post-exposure, to detect any changes in respiratory pattern or frequency.

Recognition of Irritation Responses

Tracings of the breathing patterns were recorded onto a roll of chart paper on the Gould recorder during the control period to verify that all animals had normal breathing patterns before the start of exposure. Any animal which had an abnormal frequency or pattern was replaced in the exposure chamber with a new animal and the control period was lengthened to allow the acclimation of the new mouse. The recorder pens were started at least 1 minute before the start of exposure to register any abnormal breathing pattern changes which might occur when aerosolization began. Chart recordings were obtained at various times during the exposure period, to monitor patterns and verify frequency changes. Recordings were also obtained to determine recovery information post-exposure.

As previously described by Alarie, characteristic changes in respiratory patterns can be seen in mice when exposed to sensory and pulmonary irritants. During inspiration, the change in pressure measured by the transducer is converted to an upward pen stroke on the chart recorder. Expiration is drawn as a downward stroke. As sensory irritants stimulate the trigeminal nerve in the nasal mucosa, causing closure of the glottis and an increase in laryngeal resistance, which is seen as a "braking" pattern at the beginning of expiration (Vijayaraghavan et al., 1993). (see Figure 1) This prolonged expiratory phase in effect slows the breathing frequency, a reflex action which helps to minimize the exposure of the respiratory tract to the irritant. Pulmonary irritants

stimulate the vagal nerve, producing rapid, shallow breathing. With higher concentrations of airborne chemicals, characteristic pausing after the completion of expiration may occur, thereby decreasing breathing frequency in mice. (see Figure 2) Both sensory and pulmonary irritation may result in decreases in frequency which are proportional to the exposure concentration (Alarie, 1981).

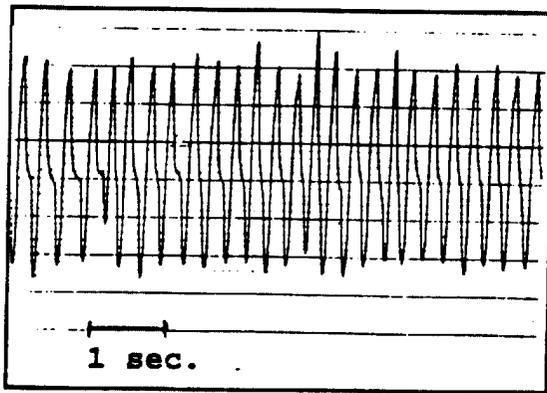


Figure 1 Sensory Irritation

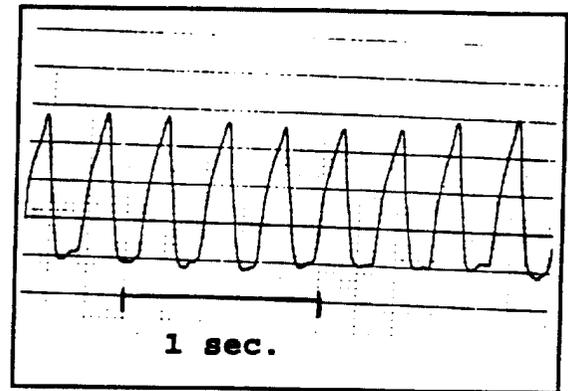


Figure 2 Pulmonary Irritation

Statistical Analysis

The maximum change in f that occurred during the 180 minute exposure was evaluated with respect to control. Significant responses were plotted as a function of the logarithm of exposure concentration. Least-squares regression analysis was then conducted to establish concentration-response relationships (i.e., testing that the slope of the line from regression analysis was significantly different from zero, $p < 0.05$). These relationships were used to calculate the exposure concentration resulting in a 50% decrease in respiratory frequency (RD_{50}) of the exposed animals.

Results

In their previous evaluation of the acute respiratory effects of aerosolized machining fluids in mice, Schaper and Detwiler (1991) noted both sensory and pulmonary irritation occurred during exposure to mists generated from neat Sample B. The present study focused on performing the same mouse bioassay, but using the individual components of this machining fluid, in an attempt to identify which component(s) was/were responsible for the irritancy of Sample B. The components of this machining fluid have varied chemical formulas, reflecting their different functions in the final product. Table 4 summarizes these results; more detailed descriptions of the component exposures are discussed below.

The manufacturing process used to produce this machining fluid involves the combination of certain compounds, which are anticipated to react with other components to produce a final "working component." For example, the manufacturer has stated that boric acid is added to the product, which should react with amines present to produce boramide (personal communication). Both boric acid (powder) and boramide (liquid) were provided by the manufacturer for testing purposes. Although these compounds may react further in the final product, it is expected that the results obtained here are representative of the irritancy potential of the original components.

Table 4 Summary of Qualitative Responses to Components of Sample B

	Sensory Irritation	Pulmonary Irritation	Plateau in Response during exposure	Recovery immediately post-exposure
Alkanol-amide #1	Yes, only later in exp. at low conc.	Yes, 1-3 hr at higher conc.	Yes, 2-3 hr	Poor
Alkanol-amide #2	Yes, 0-3 hr	Yes, 1.5-3 hr, at higher conc.	Yes, 2-3 hr	Poor, at higher conc.
Boric Acid	Yes, slight 2-3 hr	Yes, slight 2-3 hr	No significant decreases obtained	NA
Boramide	Yes, slight 0-.5 hr	No	No significant decreases obtained	NA
Petrol. Oil	Yes, 0-3 hr	Yes, .5-1 hr	Yes, 1-3hr, later with high conc.	Moderate
Sodium Sulfo-nate	Yes, 0-.5 hr	Yes, 0-3 hr	No, continues decrease in 4th hr*	Poor
Triazine	Yes, 0-3 hr, Severe	Yes, 2-3 hr at higher conc.	Yes, .5-3 hr after immediate drop in f	Poor
Potass. Soap	Yes, 0-3 hr	Yes, .75-3 hr at high conc.	Yes, 1-3 hr, later at higher conc.	Moderate. except at higher conc.
"Neat" Sample B	Yes, 0-3 hr	Yes, 2-3 hr.	Yes, 1-3 hr. later at higher conc.	Poor

*An additional exposure was continued for a 4th hour to determine if a plateau was reached.

Concentration-response relationships were developed for all components except boric acid and boramide. It was not possible to generate mists with adequate concentrations of these chemicals to produce decreases greater than 20% in respiratory frequency. Concentration-response relationships for the components are compared to Neat Sample B in Figure 3. Individual curves may be found in Appendix A and regression formulas may be found in Table 6. Using the concentration-response relationship for each component, its RD_{50} was determined. This was the concentration capable of producing a 50% decrease in f (mean of 4 mice). At the RD_{50} level, both analytic (i.e., gravimetric) and nominal concentrations were assessed. These data are presented in Table 5. An example of the calculations to obtain nominal concentrations are in Appendix B. Near the RD_{50} concentration (or achievable concentration with the boramide), particle sizing was performed. Schaper and Detwiler obtained a mass median aerodynamic diameter (MMAD) of $1.4 \mu\text{m}$ ($\sigma_g = 1.5$) using neat Sample B. The particle sizes of the components ranged from 1.12 to $1.93 \mu\text{m}$ ($\sigma_g = 1.93-2.33$) (see Appendix A for individual particle sizing figures).

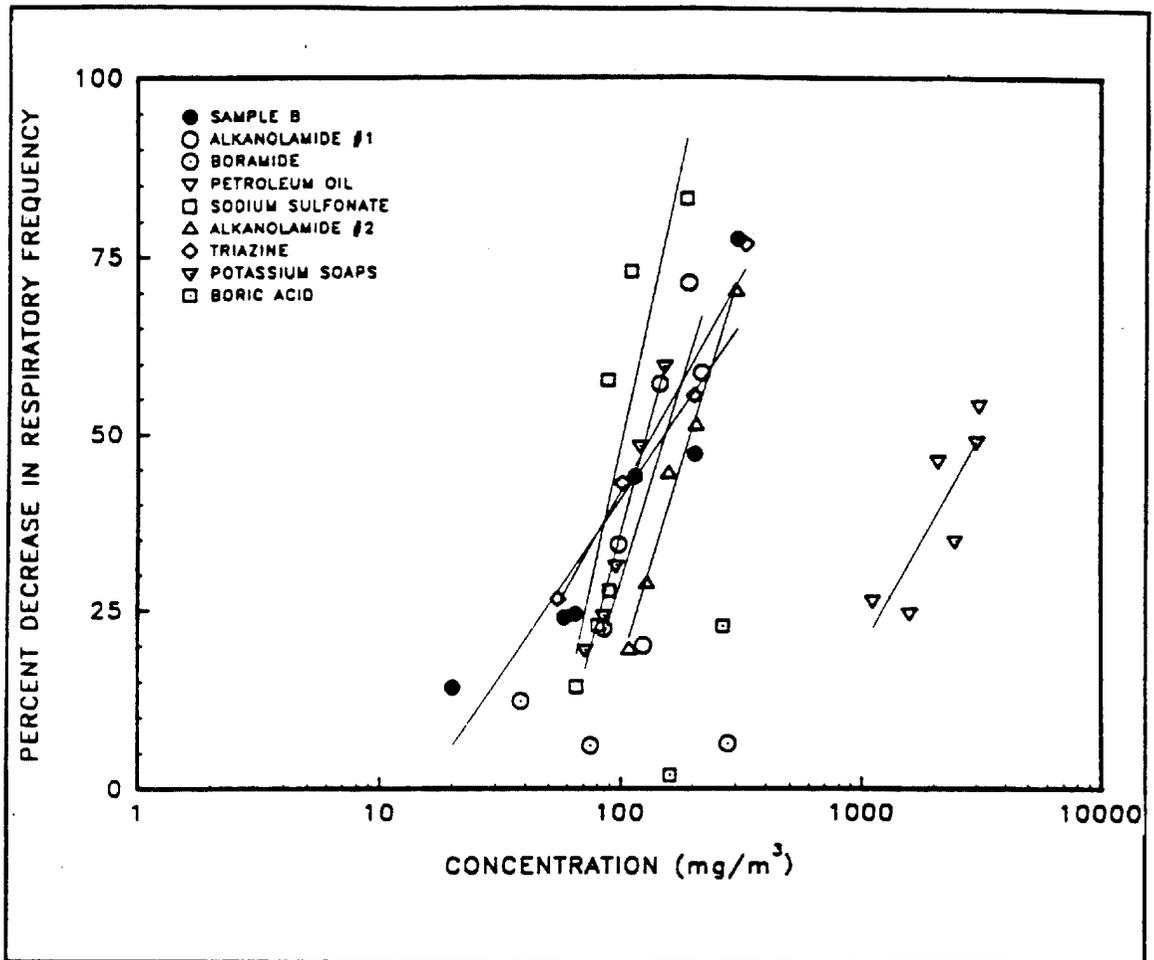


Figure 3 Concentration-Response Relationships for Sample B and its Components

Table 5 Comparison of Exposure Concentrations Determined Analytically and Nominally

Component	Gravi-metric Conc.	Nominal Conc.	% Water	Adj. Conc.
Alkan.#1	99 mg/m ³	72.8 mg/m ³	0%	72.8 mg/m ³
Alkan.#2	170 mg/m ³	183.6 mg/m ³	0%	183.6 mg/m ³
Boramide	296 mg/m ³	7400 mg/m ³	50%	3700 mg/m ³
Petrol. Oil	666 mg/m ³	1333 mg/m ³	0%	1333 mg/m ³
Sod.Sulf.	124 mg/m ³	7860.6 mg/m ³	92.5%	590 mg/m ³
Triazine	62.5 mg/m ³	232.9 mg/m ³	0%	233 mg/m ³
K Soap	70.4 mg/m ³	8467 mg/m ³	90%	847 mg/m ³

Table 6 Calculated Values for Components

	MMAD μm	σ _g	Conc. Respon Curve y = mx + b				RD ₅₀ mg/m ³	95% C.I. mg/m ³
			m	b	f	R ²		
Alkan #1	1.32	2.03	110.7	-192.45	9.17	.70	155	121-219
Alkan #2	1.45	1.89	111.5	-205.75	176.9	.98	197	180-219
Bor-amide	1.93	1.99	NA	NA	NA	NA	NA	NA
Oil	1.12	2.03	60.4	-161.56	10.25	.72	3188	2310-53500
Sod. Sulf.	1.81	1.93	157.1	-266.38	12.61	.76	103	73-172
Tri-azine	1.41	2.33	60.8	-70.85	69.2	.97	137	90-206
Potas Soap	1.82	1.99	128.8	-221.88	156.1	.98	129	119-145
Sam-ple B	1.42	1.49	49.6	-58.51	23.70	.86	154	92-260

After exposure, mice were observed for several days. For each component, respiratory frequency was monitored for at least one day after exposure to a concentration which resulted in a significant decrease in f . Only one component, Triazine, resulted in animal deaths after exposure.

Triazine

(Range of Exposure concentrations: 55 to 1619 mg/m³)

Severe sensory irritation was seen at the start of exposure, with milder sensory irritation seen even at the lowest concentration of 55 mg/m³. Immediate decreases in f were noted at the start of exposure and a plateau in response was reached in 20-30 minutes. Typically, the plateau was maintained throughout the remainder of the exposure (see Figure 4). Recovery was poor after exposure. Pulmonary irritation also occurred during and following exposures at higher concentrations to triazine.

Due to the severe adverse effects noted in these animals, more extensive follow up of animals was performed. Respiratory patterns and f were checked on the days marked, with animal deaths also noted by the numbers found in Table 7.

Table 7 Animal Mortality after Exposure to Triazine

Exposure conc.	Day 1 (exp)	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
55 mg/m ³		✓	✓	✓ 1/4	✓	✓	
103 mg/m ³	1*/4	✓	✓ 1/3	✓			
204 mg/m ³		✓ 1/4	✓ 1/3	✓	✓	✓	✓
332 mg/m ³		✓ 2/4					
1619 mg/m ³	1*/4	✓	✓ 2/3	✓	✓	✓	

*Animal euthanized after exposure due to difficulties in removing from chamber.

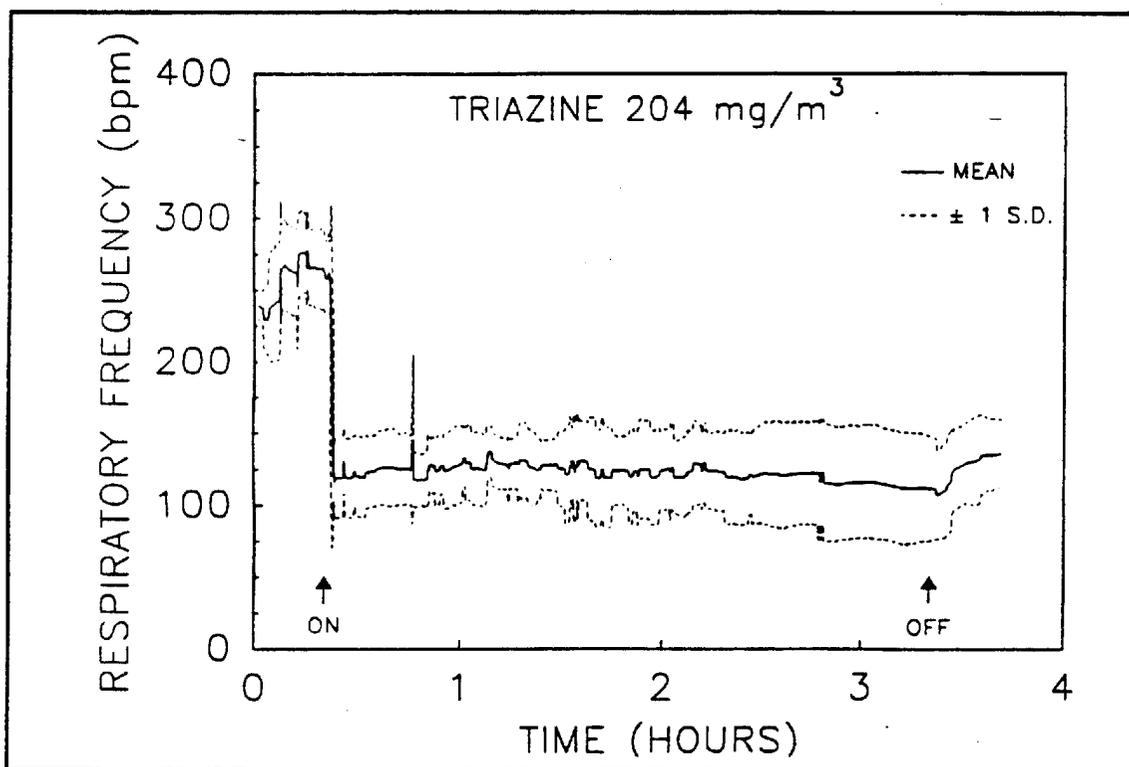


Figure 4 Triazine exposures noting immediate decrease in f , rapid development of a plateau in response, and poor recovery following exposure.

Animals were noted to have extremely swollen heads after exposure, at concentrations as low as 103 mg/m^3 . Two animals noted above with asterisks were too swollen to easily extricate them from the exposure chamber, so that tape restraints and dams had to be cut to remove the mice. Back leg paralysis due to neck damage was evident after returning them to their cages, so the animals were euthanized at that time. It is likely that nerve damage was due to removal attempts.

Twenty-four hours after exposure to Triazine concentrations as low as 103 mg/m^3 , some animals lost up to 4 g, approximately 15% of their body weight. Animals were noted to be breathing through their mouths, and were gasping, making noticeable clicking and "chirping" sounds. Sensory and pulmonary irritation patterns were seen as shown in Figure 5. This type of delayed response was not seen with any of the other components.

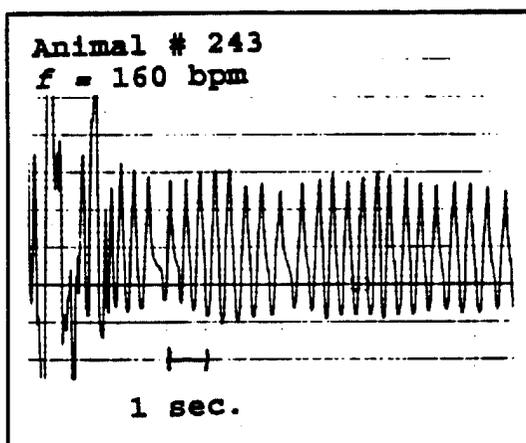


Figure 5A

Figure 5A 102 mg/m^3 Triazine exposure. Respiratory pattern seen at the beginning of exposure.

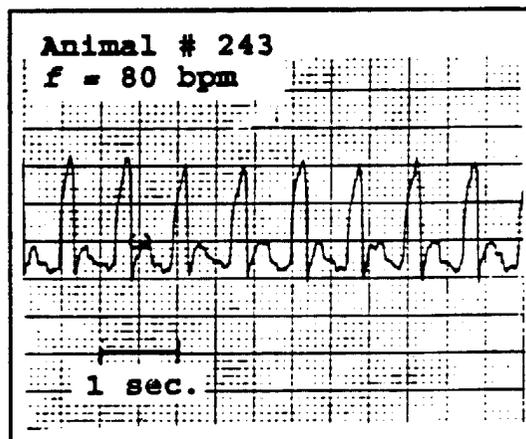


Figure 5B

Figure 5B Respiratory pattern seen 1 day after exposure.

Alkanolamides

(#1 Range of Exposure Concentrations: 86 to 219 mg/m³)

(#2 Range of Exposure Concentrations: 109 to 302 mg/m³)

The responses to the two alkanolamide components were similar, but the product designated as Alkanolamide #1 was slightly more potent than Alkanolamide #2. It did not produce sensory irritation until later in the exposure at lower concentrations, while Alkanolamide #2 produced sensory irritation immediately at the start of exposure. Once noted, sensory irritation continued throughout the exposure with both components. Pulmonary irritation also occurred later in these exposures (t = 2-3 hr), and was more severe with Alkanolamide #1 than #2. Both components produced variable effects in the animals, with one or two animals having dramatically lower breathing frequencies than the other mice, even with the lowest concentrations. Figures 6 and 7 are representative of the variability seen between animals exposed to Alkanolamide #1. In Figure 7, the variation in *f* was quite wide, as two animals had large decreases in *f*, while the other two animals had little decrease in *f*. The animals affected by this component also showed a decrease in VT. Animals exposed to Alkanolamide #2 were noted to have crystalline crusts around their eyes when removed from the exposure chamber.

Twenty-four hours after exposure (Day 2) to 208 mg/m³ of Alkanolamide #2, the animals appeared normal on gross inspection and had normal breathing patterns and frequencies when checked with plethysmography. Animals exposed to 147

mg/m³ and 219 mg/m³ of Alkanolamide #1 were also followed after the exposures. At Day 2, all animals physically appeared normal, and respiratory patterns and frequencies were essentially normal. Two mice from the 219 mg/m³ exposure group had slightly decreased *f*, so it was decided to follow that group further. These animals were checked for an additional four days. Frequencies during that time period were elevated, to approximately 325 breaths/min., and slight pulmonary irritation was seen at Days 5 and 6 in one animal.

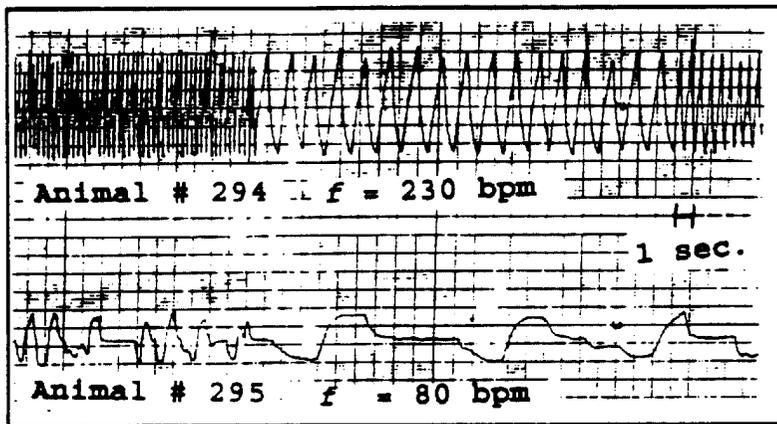


Figure 6 Alkanolamide #1, Variation in patterns of 2 mice exposed to 147 mg/m³.

Boramide/Boric Acid

(Range of Boric Acid Exposure Concentrations: 161 to 268 mg/m³)
(Range of Boramide Exposure Concentrations: 38 to 279 mg/m³)

The boramide fluid provided by the manufacturer resembled a thin oil, and when used with the Pitt #1 generator produced a thick, white foam that clogged the generator. Dilution of the product with deionized water improved its tacky consistency only slightly. The maximum achievable concentration using the Pitt #4 generator was 279 mg/m³, which produced no decrease in respiratory frequency. No sensory or pulmonary irritation was noted. The animals were followed for four days after exposure. Frequencies were slightly increased on Days 2-4, but no abnormal patterns were seen.

Boric acid also presented a problem with aerosol generation. A saturated solution (10% w/v) was prepared, however the maximum concentration achieved using the Pitt #4 generator was 268 mg/m³. Attempts to increase the solubility resulted in a change in pH, so the solution was heated in an attempt to maximize the soluble amount of chemical in the test samples. No sensory irritation was noted at the beginning of exposure, but after 2 hours, slight sensory irritation was noted. Slight pulmonary irritation was also noted in a few animals later in the exposures. No decrease in *f* was seen at the maximum achievable concentration. The animals' eyes were encrusted with boric acid crystals after the exposures. Days 2 and 3 post-exposure, frequencies averaged >300 breaths/min. versus approximately 250 breaths/min. seen during control.

Pulmonary irritation was noted with short pauses. Because of these difficulties with boramide/boric acid, it was not possible to generate concentration-response relationships.

Potassium Soap

(Range of Exposure Concentrations: 72 to 154 mg/m³)

Sensory irritation was seen at the start of exposure and continued throughout the exposure period. Decreases in VT occurred after as little as 30 min. of exposure, at concentrations greater than 121 mg/m³. Pulmonary irritation was minimal, with decreased *f* due to severe sensory irritation. Recovery immediately post-exposure was relatively rapid, except for the highest concentrations. Some of the animals were noted to have swollen heads and irritated eyes. At higher concentrations, the their facial fur appeared damp and coated with soap. On Day 2, the animals had normal patterns and frequencies when checked in the plethysmographs.

Petroleum Oil

(Range of Exposure Concentrations: 1600 to 3114 mg/m³)

Sensory irritation was noted at the start of exposure and did not dissipate until exposure was completed. Pulmonary irritation also developed rather quickly, usually after about 30 minutes. This component produced similar decreases in *f* of the four animals at each concentration. Recovery after the completion of exposure was moderately rapid. The animals were noted to have swollen heads, and were very oily when removed

from the chamber. The animals began to groom themselves after the exposure. They were grossly observed for several days following exposure, but were not checked in the plethysmographs after exposure.

Sodium Sulfonate

(Range of Exposure concentrations: 66 to 190 mg/m³)

Sensory irritation was seen at the start of exposure, but dissipated after approximately 30 min. Frequencies increased at the start of exposure and at concentrations less than 80 mg/m³, many animals did not decrease *f* below their baseline rates. Individual animal responses varied, producing a wide range in the standard deviation of *f*. At higher concentrations, *f* began to decrease after approximately 30 minutes and continued to decrease, often without reaching a plateau. These responses are shown in Figure 8. Pulmonary irritation was severe at higher concentrations, with pauses sometimes seen at the start of exposure. VT gradually decreased at higher concentrations. There was a great deal of variability in the responses of the mice, noted especially with the concentrations greater than 80 mg/m³. The animals' eyes were noted to be irritated, and "glazed" with a film upon removal from the exposure chamber. Because sodium sulfonate did not produce a plateau in response after the three hours of exposure, a four hour exposure was performed using an exposure concentration of 80 mg/m³. As seen in Figure 9, a plateau in response did not occur even after 4 hours of exposure.

The mice appeared wet and their fur was ruffled for 48 hours after the exposure. Most animals lost weight, up to 2 g, by Day 2. For most animals, f was slightly lower than normal Days 2 and 3, however the patterns appeared relatively normal. One mouse which had maintained a higher breathing frequency for most of the exposure to 189.7 mg/m^3 was severely affected. By Day 3 post-exposure, this animal lost 3 g and was breathing at approximately 100 breaths/minute. Its respiratory pattern was relatively normal, and there was no evidence of sensory or pulmonary irritation.

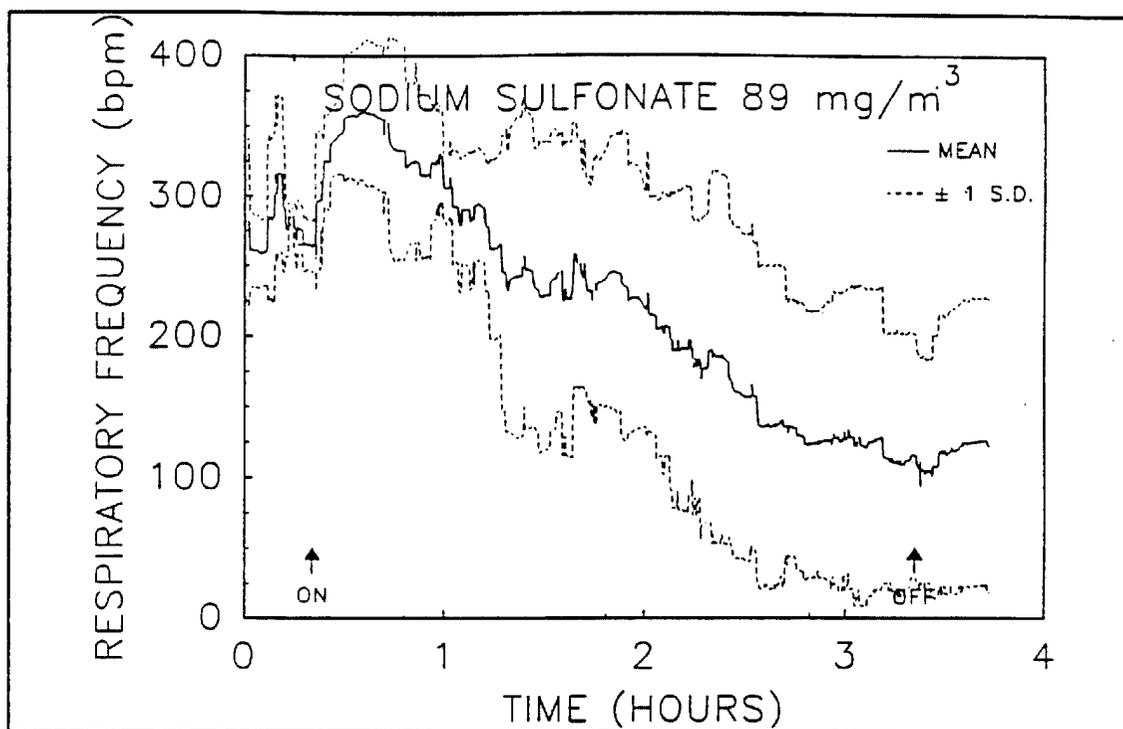


Figure 8 89 mg/m³ Sodium sulfonate, 3 hour exposure

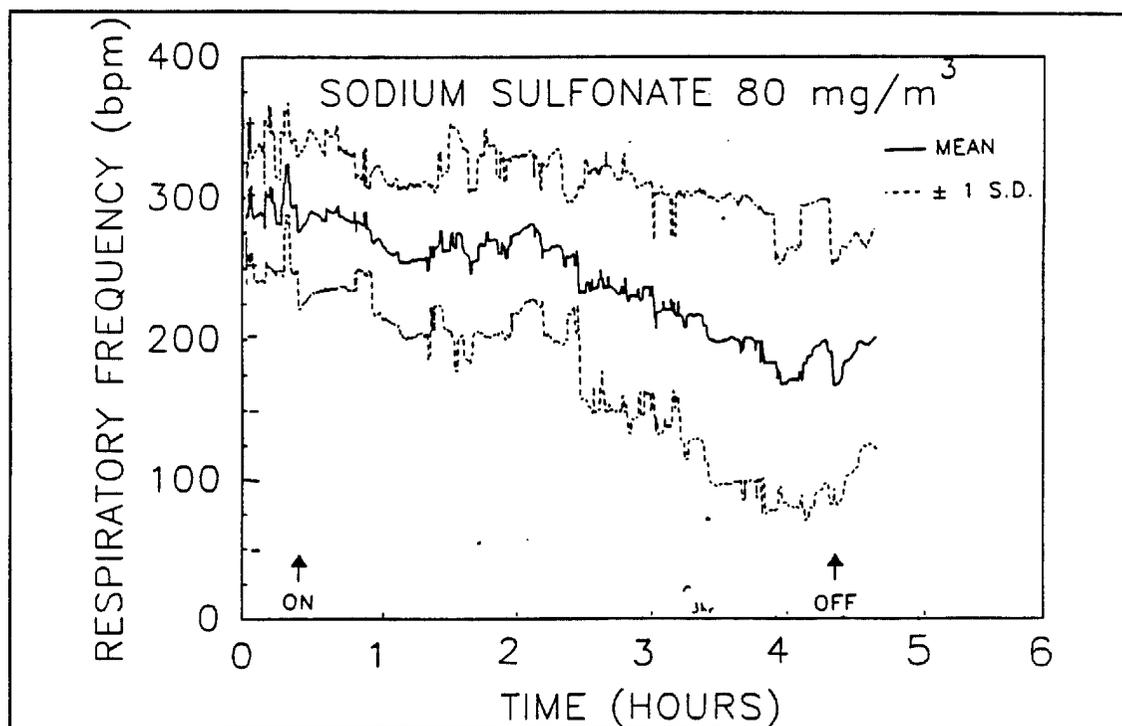


Figure 9 80 mg/m³ Sodium sulfonate, 4 hour exposure

Discussion

Review of Component Actions

In a previous study, Schaper and Detwiler(1991) identified that Sample B possessed sensory and pulmonary irritating properties in both its neat form and an in use sample. The present study has identified that all eight component samples (including boric acid and boramide) may produce sensory irritation. In addition, all components except boramide were shown to produce some pulmonary irritation patterns. Again, as the highest concentration of boramide which could be generated was 280 mg/m^3 , it may be possible that this component could produce pulmonary irritation at higher concentrations. -EUC

While neat Sample B had an RD_{50} value of 154 mg/m^3 , there are five components of this metalworking fluid which also have RD_{50} values in the $100\text{-}200 \text{ mg/m}^3$ range- the two alkanolamides, sodium sulfonate, triazine and potassium soap. The main effect of these fluids was on f , with rapid decreases in f especially noted with triazine. Sodium sulfonate and the alkanolamides also caused a noticeable decrease in VT. Recovery immediately post-exposure was poor with those five components noted to have the lower RD_{50} values. It may be concluded that these five components all contributed to the irritancy of Sample B.

Petroleum oil comprises 20% of the final Sample B product. Identified as the least potent component with an RD_{50}

value of 3188 mg/m³, it probably did not contribute to the irritancy of Sample B. This agreed with the findings of Shoskes et al., (1950) who exposed mice to 4500 mg/m³ of liquid petrolatum and found no inflammatory changes in the lungs of exposed mice which were sacrificed at various times post-exposure. Stained sections of lung tissue indicated that the oil had been phagocytized within 48 hours after exposure. Costa and Amdur (1979) concluded that exposure to straight oil mists was not irritating after finding that guinea pigs exposed to these mists had little change in *f* or VT. Schaper and Detwiler (1991) found that of the seven neat fluids tested in their comparison of machining oils, the straight oil product had the highest RD₅₀, extrapolated to 325,000 mg/m³.

This study was primarily focused on the acute respiratory effects of the metalworking fluid components. While one or more groups of animals were followed for at least 24 hours after exposure to the components, long term effects on the animals were not investigated. Alkanolamide #1, boric acid and boramide produced increased *f* after the exposures, while sodium sulfonate caused decreased *f* post-exposure. Triazine produced such severe lung damage in eight out of the twenty exposed animals that the animals died within 3 days after exposure to concentrations as low as 55 mg/m³. Other exposed animals also exhibited the "chirping" and "clicking" breathing noises, but did not die after the single exposure. Although triazine only comprises 3% of the final product, further evaluation of its irritancy potential should include long term

effects involving multiple exposures and review of lung histopathology. However, since most of the surviving mice already appeared extremely ill, any further exposure would probably have resulted in increased animal mortality.

In their original evaluation of metalworking fluids, Schaper and Detwiler (1991) removed the lungs of mice after exposure to the neat metalworking fluids. They noted that there was little change in lung weight, lung volume displacement or pulmonary pathology in animals sacrificed immediately following exposure to any machining fluid. Only at 24 hours after exposure was there evidence of mild to moderate interstitial pneumonitis and bronchopneumonia. These findings supported the observed respiratory responses which showed mostly sensory irritation and some evidence of pulmonary irritation, particularly after several hours of exposure. They did not find extensive hemorrhage or edema, which was consistent with the results of Lushbaugh et al. (1950), Shoshkes et al. (1950), Wagner et al. (1964), Stula and Kwon (1978), and Selgrade et al. (1990). It would be of interest to examine the lung histopathology caused by the triazine exposures, as the mice produced such unusual clicking noises when breathing. The pulmonary damage caused by the triazine exposures must have led to much more extensive lung damage than that found by Schaper and Detwiler, but because of its relatively small percentage in the final product, its effects were not as extensive.

The use of biocides, such as triazine, have been regulated by the Environmental Protection Agency (EPA) under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Considered "economic poisons" because they are sold for their toxicity, they are used to protect metalworking fluids from the overgrowth of bacteria and fungi. Microorganisms cause the deterioration of fluids, corrosion of work pieces and physical plugging of flow lines. The appearance of slime and the generation of foul odors associated with bacterial contamination also affects the perceptions of workers, even though they have not been shown to be at increased risk of infection from the presence of bacterial overgrowth. Guidelines for the use of biocides have been changed in the past 20 years to assure maximum worker safety, however, biocide usage is often dependent of the type of process being performed. Toxicity and corrosivity data are traditionally based on the most concentrated product usage; since most machining fluid concentrates are diluted to various levels when used, the prediction of biocide concentration is nearly impossible to uniformly predict. There is a tendency for producers to refer to biocides as fluid restorers, conditioners, deodorants, or other terms which do not imply antimicrobial activity. One of the most popular types of biocides in terms of total sales are compounds known as formaldehyde condensate products. These products are produced by reacting formaldehyde with one or more substituted alkylamines to yield triazine. The release of formaldehyde

during use is presumed to occur at the microbial cell boundary, as the original product is hydrolyzed at the more favorable pH. These fluids are considered extremely stable at the alkaline pH of metalworking fluids, so that formaldehyde release should not be readily detected (Holtzman and Rossmore, 1977). The potential toxicity of released formaldehyde could still be considered. If formaldehyde is indeed released from a biocide, this would produce sensory irritation. Its RD_{50} is 38.83 mg/m^3 , and it is a potent, rapid acting sensory irritant (Kane and Alarie, 1977).

Mixed sensory and pulmonary irritant effects were seen not only upon exposure to neat Sample B and many of its individual components, but also with other synthetic and semisynthetic machining fluids (Schaper and Detwiler, 1991). A machining fluid such as Sample B is a complex mixture of different products. The manufacturer combines certain compounds, assuming that they will react to form the intermediate compounds, some of which have been provided for this study as components of Sample B (personal communication). CAS numbers and component percentages were obtained from the original Sample B Material Safety Data Sheet (MSDS). However, MSDSs for some components were not provided, perhaps because several of the components are the result of chemical reactions, and not a marketed chemical commodity. Due to the uncertainty of these chemical reactions and absence of other information on the MSDS, such as proprietary ingredients, it may not be valid to assume that the irritancy response of a

commercially available machining fluid product will necessarily be equal to the additive irritancy potentials of its listed components.

Determination of Sensory Irritation

In 1992, Nielsen and Alarie described structure-activity relationships of sensory irritants and reviewed sensory irritation mechanisms. During the stimulation of trigeminal nerve endings in the nasal mucosa by airborne chemicals, the reflex reactions associated with sensory irritation (decreased respiratory frequency, characteristic prolongation of the expiratory phase, etc.) are noted (Alarie, 1981). Two different mechanisms have been identified- one in which the substances react chemically with receptors and the other in which the substances physically adsorb to the receptors lining the upper respiratory tract. The animal bioassay described by Alarie in 1966 uses these concentration-response relationships to determine the RD_{50} . When mixtures of chemicals are evaluated using the mouse bioassay, receptor site interactions may affect the predicted outcome.

Kane and Alarie (1978) evaluated sensory irritation from acrolein-formaldehyde mixtures to identify independent versus competitive interaction (agonism) responses to sensory irritants. It has been shown that the response, R , is proportional to the number of receptors sites filled in the trigeminal nerve endings (Alarie, 1973). If two sensory irritants act at equivalent, independent receptor sites, the response to

a mixture would be expected to be the sum of the responses to each component. If the two irritants act at the same type of receptor site, the response would be less than the sum of the two individual responses.

Nielsen et al. (1988) recognized that while the additivity model was frequently used for industrial exposures to mixtures of chemicals, there were few data to support that assumption. In their investigation of cumene and propanol mixtures, representative of alkylbenzenes and alcohols found in industrial settings, competitive agonism was identified. Their work and that of Kane and Alarie (1978) lend experimental support to the formula used by ACGIH for recommending exposure levels for the evaluation of mixtures. However, for this formula to be valid, all of the chemicals in the mixture must exert the same biological effect (ACGIH, 1993). As the components tested in this study did not exert the same effects, and the effects may not be additive, this model may not be satisfactory to determine a safe level of exposure for Sample B.

Determination of Pulmonary Irritation

The lower respiratory tract includes all structures below the larynx, down to the alveoli. Vagal type-C nerve endings may be stimulated directly by inhaled chemicals known as "direct-acting vagal nerve ending stimulants," which quickly induce reflex effects (Vijayaraghavan et al., 1993). Recovery is rapid after the cessation of exposure. Pulmonary irritants also stimulate the vagal nerve endings, however this action is

slower, and may produce an inflammatory reaction, edema or congestion. The respiratory rate initially increases, but tidal volume decreases. With pulmonary irritants, this respiratory pattern will permit greater absorption of the chemical on the conducting airways, thus reducing the amount reaching the alveoli (Alarie, 1973). Chemicals capable of inducing both sensory and pulmonary irritation stimulate receptors in both the upper and lower respiratory tract, however, sensory irritation is frequently an immediate reaction and may mask pulmonary irritant response. Careful monitoring of respiratory patterns is essential to detect delayed pulmonary irritation. To confirm pulmonary irritation properties, animals may be exposed via tracheal cannula, which will eliminate trigeminal nerve stimulation, thus only pulmonary effects will be detected.

Neilsen and Yamagiwa (1989) exposed male Ssc:CF-1 mice to known airway irritating aliphatic amines. They concluded that the quick recovery of their tracheal-cannulated animals immediately post-exposure suggested that the compounds activated the pulmonary receptors directly. They noted that if the pulmonary irritation response had been due secondary effects, (e.g., edema or inflammation) a slower normalization of the respiratory rate would have been expected.

Weyel et al. (1982) and Weyel and Schaffer (1985) identified the mixed sensory and pulmonary irritation properties of isocyanates. Noting that the use of the mouse bioassay in predicting exposure limits for humans was based on

sensory irritation, they recommended dividing the RD_{50} for pulmonary irritants by 60 to suggest occupational exposure limits. This extrapolation of the RD_{50} value provides a smaller acceptable concentration level than the $.03 \times RD_{50}$ calculation used for sensory irritants, reflecting the greater potential for lung damage from exposure to pulmonary irritants.

Cumulative and Sensitizing Effects

Many low molecular weight chemicals (LMWCs) (M.W. < 1000) are recognized as causative agents of occupational asthma (Agius et al., 1991). Allergic asthma can result when these reactive substances haptenate carrier proteins to form immunogenic conjugates, which then induce specific allergic antibodies (Gauggel et al., 1993). Agius et al. (1991) also noted that animal exposures to low molecular weight substances have had limited success in reproducing these effects seen in man. Gauggel et al. (1993) proposed an assessment process for evaluating the allergenic potential of LMWCs which includes evaluating structure-activity relationships, to determine whether or not the LMWC has the ability to covalently derivatize protein carrier molecules. They agreed however that at this time, only *in vivo* testing can provide definitive and appropriate guidelines for establishing safe exposure levels in the workplace.

Detwiler and Schaper (1991) evaluated four of the machining fluids used in their original study to determine if

the effects of repeated exposure were cumulative or sensitizing. Groups of mice were exposed to each fluid at its RD_{50} on Days 1, 2, 3, 4, 5 and 14 for 3 hours/day. They found that the same relative decrease in respiratory rate was evoked each day; thus there was no evidence of a cumulative effect. In its final formulation, Sample B contains 54% water and 3-5% of each of the five components identified as the most irritating. It is possible that one or more of the individual components may produce cumulative effects, but in the final product this effect may be minimized due to the dilution factors. The speculations of Drasch et al. in 1974 that chronic exposure to oil mist at concentrations between 40-150 mg/m^3 protected workers from the adverse effects of smoking also raises the possibility that the oil present in Sample B helped to protect the lungs of the mice from the more harmful effects of the other components.

Continuing their 1991 study, Detwiler and Schaper also exposed groups of guinea pigs to each fluid at its RD_{50} on Days 1, 2, 3, 4, and 5 for 30 minutes/day. Similar exposures were conducted on Days 19, 33, 47, 61 and 75. While no pulmonary effects were observed on Days 1-5 or Day 19, bronchoconstriction occurred in 2 out of 4 animals exposed to neat Sample B on Days 33-75. They concluded that the semi-synthetic fluid may contain a sensitizing ingredient. Further evaluation of these components would be necessary to identify any sensitizing compounds.

Development of TLVs

The mouse bioassay has been used extensively to develop safe exposure limits for humans. Alarie (1973) has shown that the mouse bioassay is predictive of human response, and the bioassay has been validated. Alarie (1973), Kane et al. (1979) and Alarie et al. (1980) provided "calibration" of the bioassay by showing that humans exposed to a chemical at its RD_{50} would experience intolerable burning of the eyes, nose and throat. They predicted that slight irritation would occur at $0.1 \times RD_{50}$ and minimal or no effect would occur at $0.01 \times RD_{50}$. While values in this range would be acceptable for human exposure, $0.03 \times RD_{50}$, the midpoint between 0.1 and 0.01 on the logarithmic scale, has often been used to establish threshold levels. In 1993, Schaper developed a database for sensory irritants which included 295 airborne materials whose sensory irritating properties had been evaluated using the mouse bioassay. Of the total of 154 RD_{50} values obtained in male mice of various strains, 89 chemicals had established TLVs. Using the approach previously described by Alarie (1981) and Alarie and Luo (1986) to correlate the logarithms of the 1991 TLV values and the logarithms of $0.03 \times RD_{50}$ values, the correlation coefficient, R^2 , was 0.78. Therefore, it appears that the relationship between the TLV and $0.03 \times RD_{50}$ is well established. On the basis of sensory irritation alone, occupational exposure limits were suggested for each component (see Table 8). For pulmonary irritants, a relationship between the mouse RD_{50} and TLV has not been demonstrated. Although

Weyel et al. (1985) have recommended that the RD_{50} be divided by 60 to protect workers from pulmonary irritating properties of chemicals, this is a "best guess" at this time. Because the components also acted as pulmonary irritants, the RD_{50} values divided by 60 have also been provided in Table 8.

Table 8 Calculated Exposure Limits for Components

Component	RD_{50}	$.03 \times RD_{50}$	$1/60 \times RD_{50}$
Alkanolamide #1	155 mg/m ³	4.7	2.6
Alkanolamide #2	197 mg/m ³	5.9	3.3
Petroleum Oil	3188 mg/m ³	95.6	53.1
Sodium Sulfonate	103 mg/m ³	3.1	1.7
Triazine	137 mg/m ³	4.1	2.3
Potassium Soap	129 mg/m ³	3.9	2.2
Sample B	154 mg/m ³	4.6	2.6

Threshold Limit Values for Mixtures

The ACGIH provides guidelines for determining TLVs for mixtures in Appendix C, noting that when two or more hazardous substances which act upon the same organ are present in a mixture, their combined effects must be given primary consideration. If there are no data to suggest otherwise, the effects may be considered additive. That is, if the sum of:

$$[C_1]/TLV_1 + [C_2]/TLV_2 + \dots [C_n]/TLV_n$$

exceeds unity, then the threshold limit of the mixture has been exceeded. Unfortunately, there are no TLVs established for these components of Sample B, so a simple calculation can not be used to evaluate if the percent concentrations of components exceed current limits.

Using the figures for pulmonary irritation calculated in Table 10 in the above additive formula results in a recommended TLV of 5.02 mg/m³, without the inclusion of a figure for boramide/boric acid. Since exposure concentrations of 280 mg/m³ of either compound did not produce a decrease in *f*, a figure calculated from extrapolation to an RD₅₀ value would only decrease this TLV by a minute quantity. The percent of each component present in the final product was adjusted to reflect the test sampling conditions which would not include water present in the final formulation. This value agrees with the previously proposed limit of 2.6 mg/m³ for neat Sample B (Schaper and Detwiler, 1991).

Table 9 Calculation of TLV Using ACGIH Mixture Formula

Contribution from component	Σ % component* [Sample B] est.TLV for component
Alkanolamide #1	.109x/2.6 .04192
Alkanolamide #2	.109x/3.3 .03303
Petroleum Oil	.435x/53.1 .00819
Sodium Sulfonate	.065x/1.7 .03824
Triazine	.065x/2.3 .02826
Potassium Soap	.109x/2.2 .04955
Boramide	.109x/unknown
	.199x = 1
	x = 5.02

Determination of Concentration-Analytical versus Nominal

When suggesting exposure limits for humans to prevent irritation from machining fluid mists, it is important to consider that airborne exposure concentrations in the work place are generally assessed via filter sampling and gravimetric analysis. With the large proportion of water that is present in the synthetic and semisynthetic fluids even before dilution to working concentrations, water mist and water vapor will be formed during industrial use. As discussed under Methods, this did not present a problem in the laboratory due to aerosol generation techniques. However, as seen in Table 5, gravimetrically and nominally determined concentrations varied widely, especially with components that required dilution in water for aerosol generation. The calculations used to determine these concentrations can be

found in Appendix B. Many additives, such as amines, have a reasonably high vapor pressure and will be volatilized during plant operations (Schaper and Detwiler, 1991). As these airborne materials will not be captured using filter sampling techniques, it may be more appropriate to utilize other methods for exposure assessment.

Recommendation of Exposure Limits

In 1989, the UAW/GM Occupational Health Advisory Board recommended that exposure levels for metalworking fluids not exceed 0.5 mg/m^3 total particulate (Jackson, 1992). Schaper and Detwiler (1991) have suggested an exposure limit of 2.6 mg/m^3 for Sample B, based on their determination of an RD_{50} of 154 mg/m^3 . If the product were used undiluted at this suggested TLV level, the 3% contribution attributable to triazine would generate concentrations equivalent to $.078 \text{ mg/m}^3$. The 1993 TLV for formaldehyde is 0.37 mg/m^3 , therefore, if triazine produced an equivalent amount of formaldehyde, this level would not exceed its TLV. An additional safety factor is involved- the manufacturer has stated that this product should be used in at least a 1:20 dilution and that up to 1:200 dilution may be used in some processes. At the working concentration, mists to which workers would be exposed would be well within acceptable levels.

This information obtained from this study only reflects one-time exposures to these compounds. As seen with triazine, harmful effects may not be apparent during the exposure

period. The potential for carcinogenesis or cumulative effects have not been evaluated in this limited study.

The universal concerns of workers about lifetime exposure to poorly characterized products are valid. Material Safety Data sheets provide little guidance, because the effects of many of these components have not been well studied. Very little data are available on the chemical nature of the components. Although some skin effects have been documented, the effects of inhalation have only been assessed on a limited basis. Many of the epidemiological investigations of exposed workers have not evaluated potential variables, such as smoking history, exposure concentration-years, or specific fluid types in their analyses.

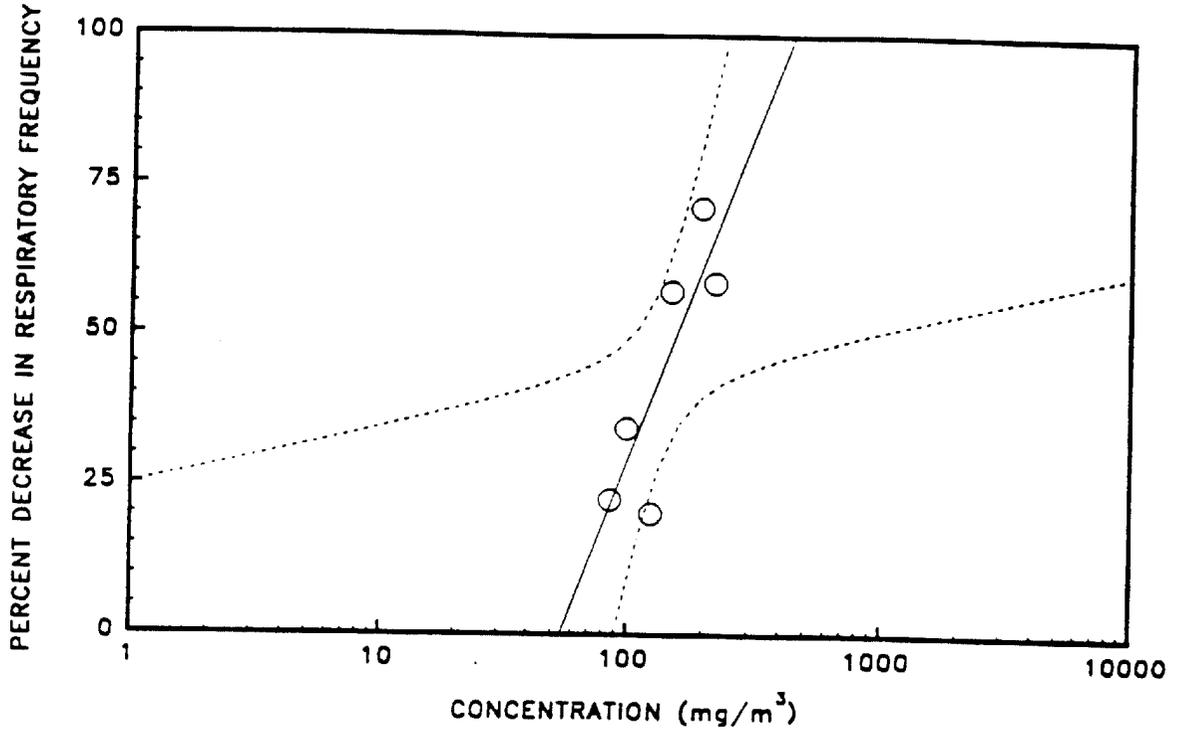
Realistically, it will not be feasible to suggest exposure limits for every individual product, such as Sample B. Although Schaper and Detwiler (1991) have evaluated 10 metalworking fluids, only two of these products were in the synthetic or semisynthetic classification. Sample A, the synthetic fluid was relatively more potent than Sample B, with an RD_{50} of 119 mg/m^3 . It is evident that these newer formulations are more potent than the older products which contained more petroleum oil, therefore lowering the TLV for the synthetic/semisynthetic products is prudent. Unfortunately, in the absence of additional data on other products, we will have to consider the RD_{50} values of only two fluids as representative of their class. The TLVs suggested by Schaper and Detwiler for Samples A and B ($2.0, 2.6 \text{ mg/m}^3$) are more protec-

tive of worker safety than the current TLV of 5 mg/m³. Although the TLV calculated using the pulmonary irritation figures determined in this study in the mixture formula (5.0 mg/m³) would lend support to maintaining the current limit, this additive model should not be used with mixed biological effects. The proposed TLV values calculated by Schaper and Detwiler (1991) using the commercially available products also account for any chemical interactions between components, providing better guidance on actual workplace exposures. Due to the magnitude of the work force exposed and the potential for adverse effects from newer chemical compounds, perhaps the exposure limit should be lowered to less than 2 mg/m³ for synthetic and semisynthetic machining fluids pending further evaluation.

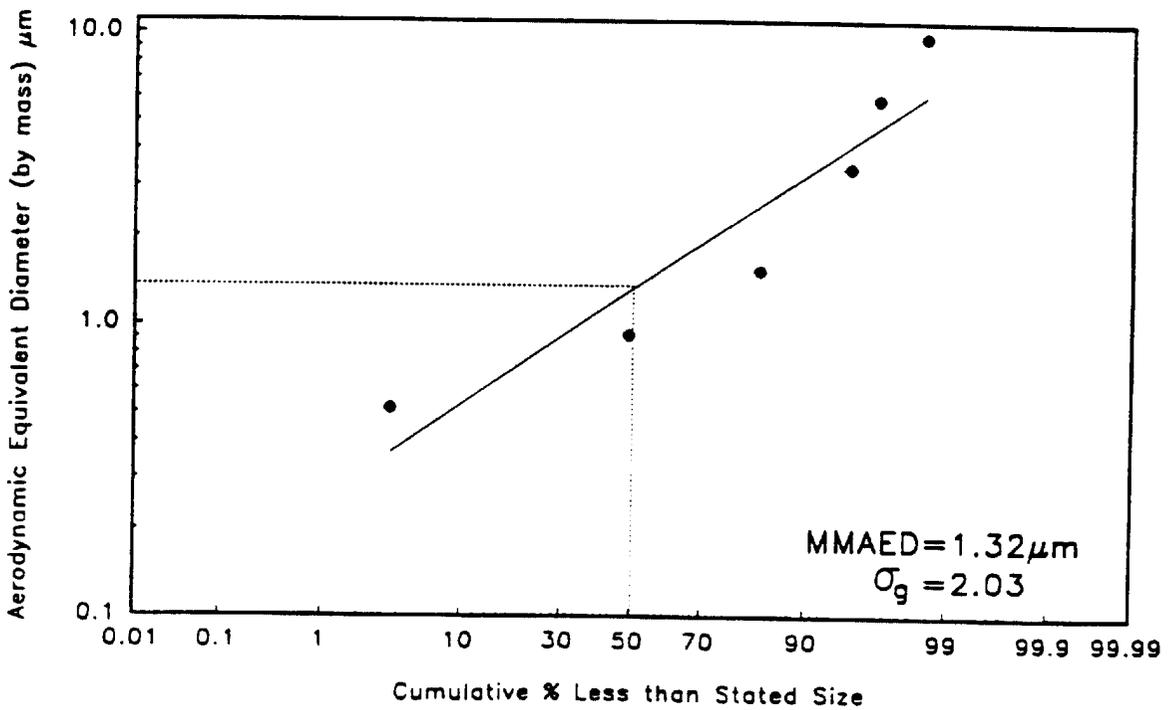
APPENDIX A FIGURES

ALKANOLAMIDE #1

Concentration-Response relationships used to calculate RD_{50} including 95% C.I.

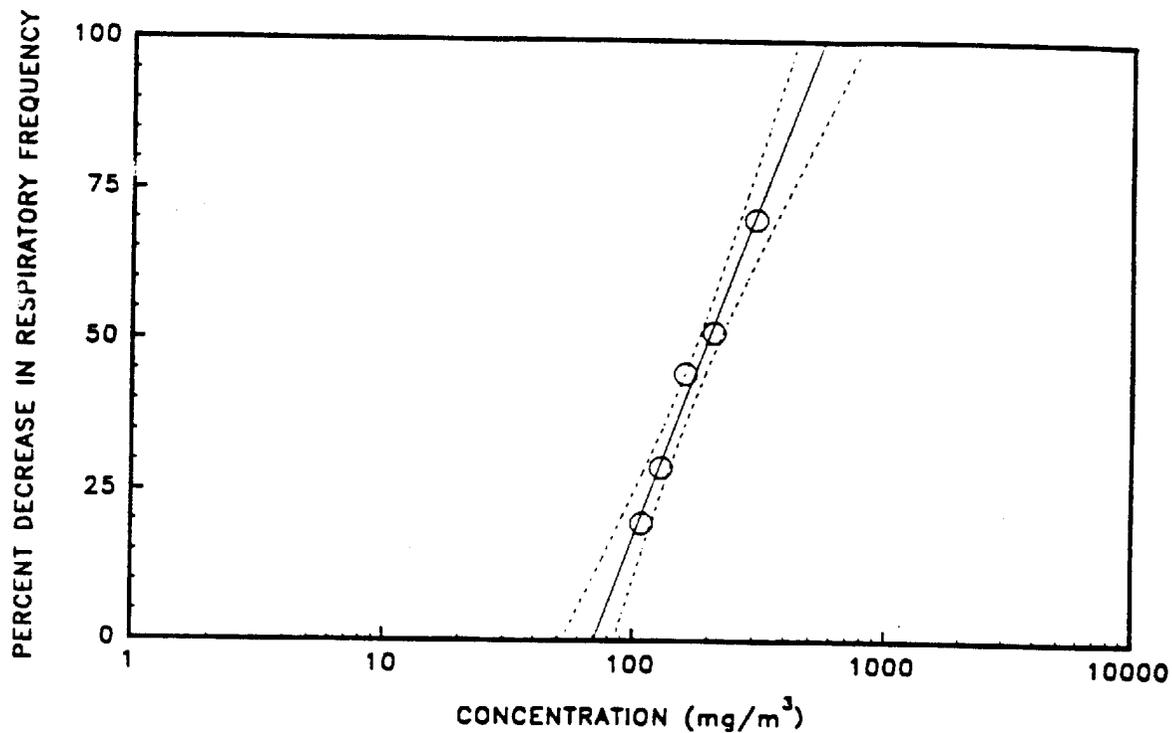


Particle Sizing

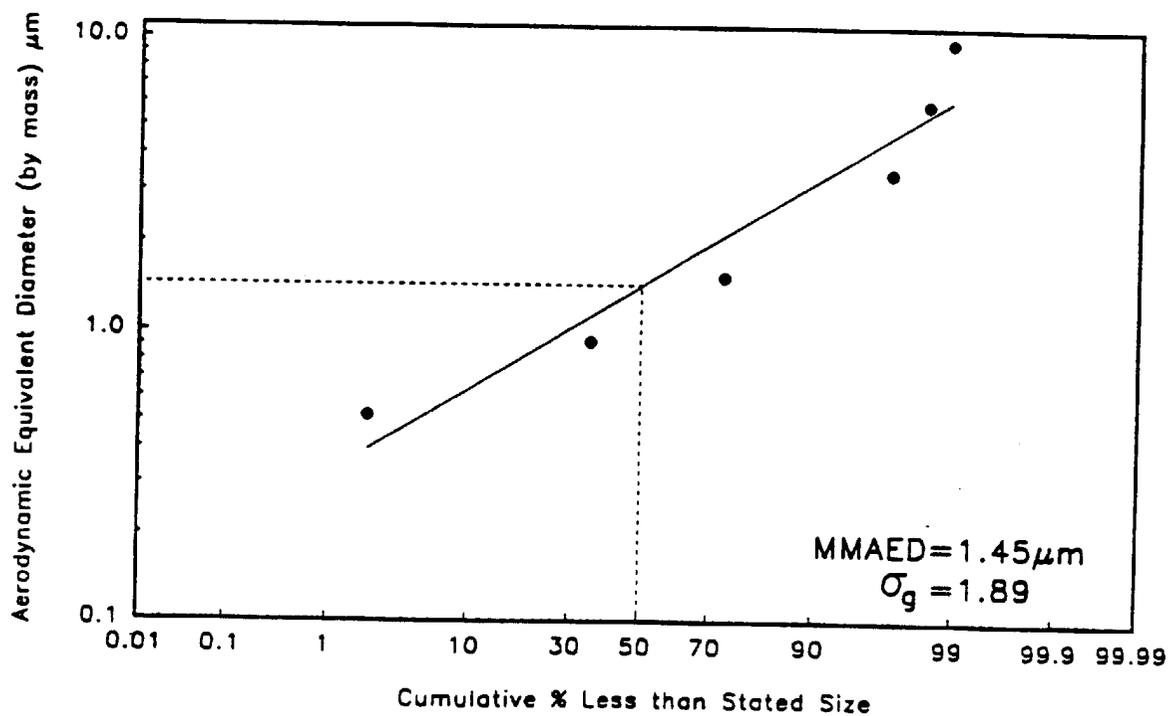


ALKANOLAMIDE #2

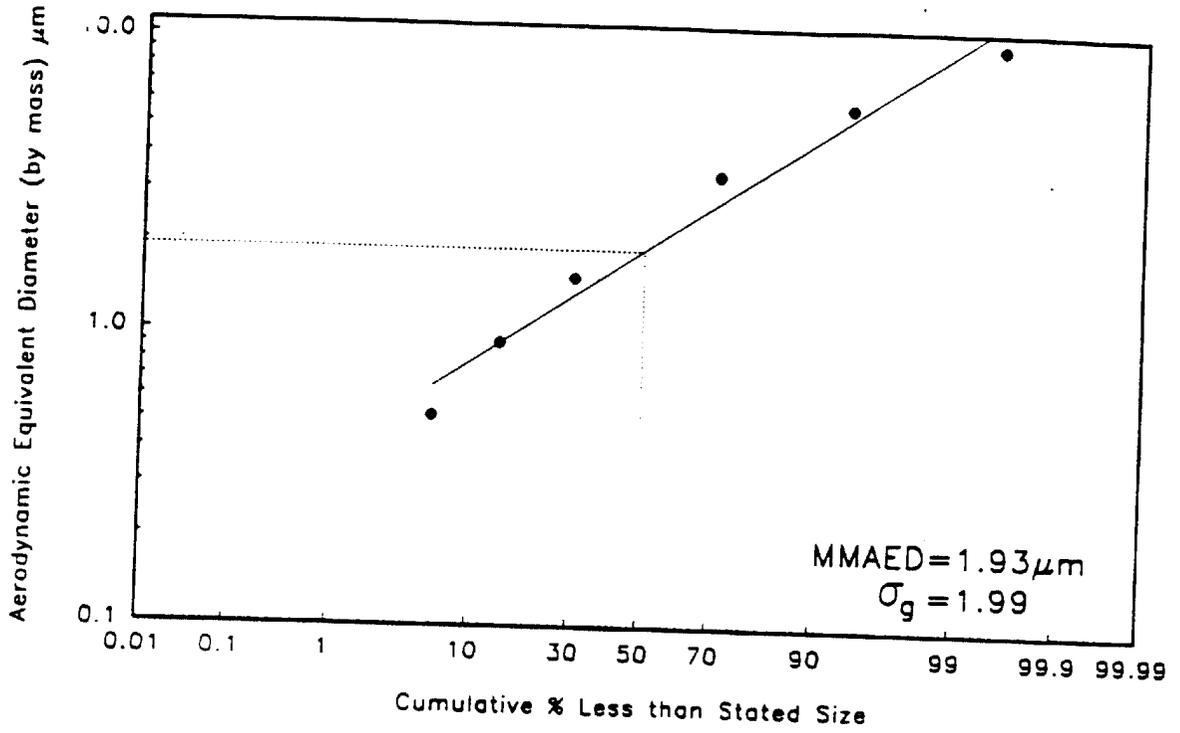
Concentration-Response relationships used to calculate RD_{50} including 95% C.I.



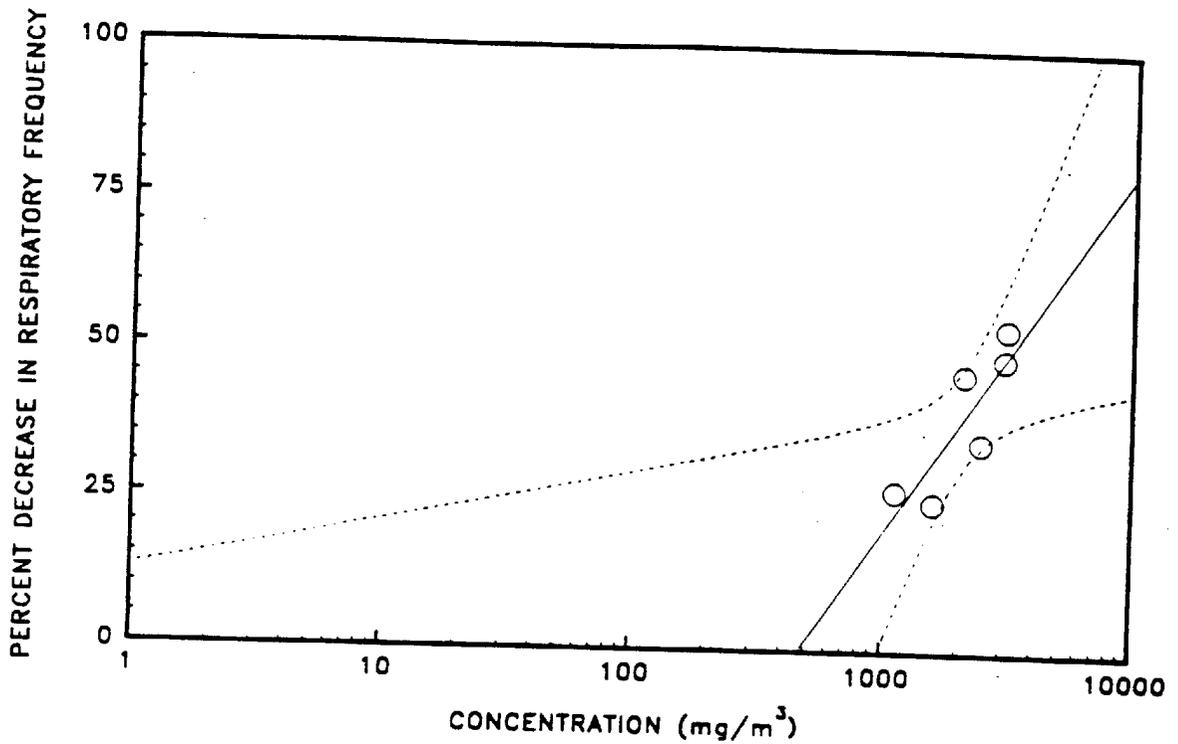
Particle Sizing



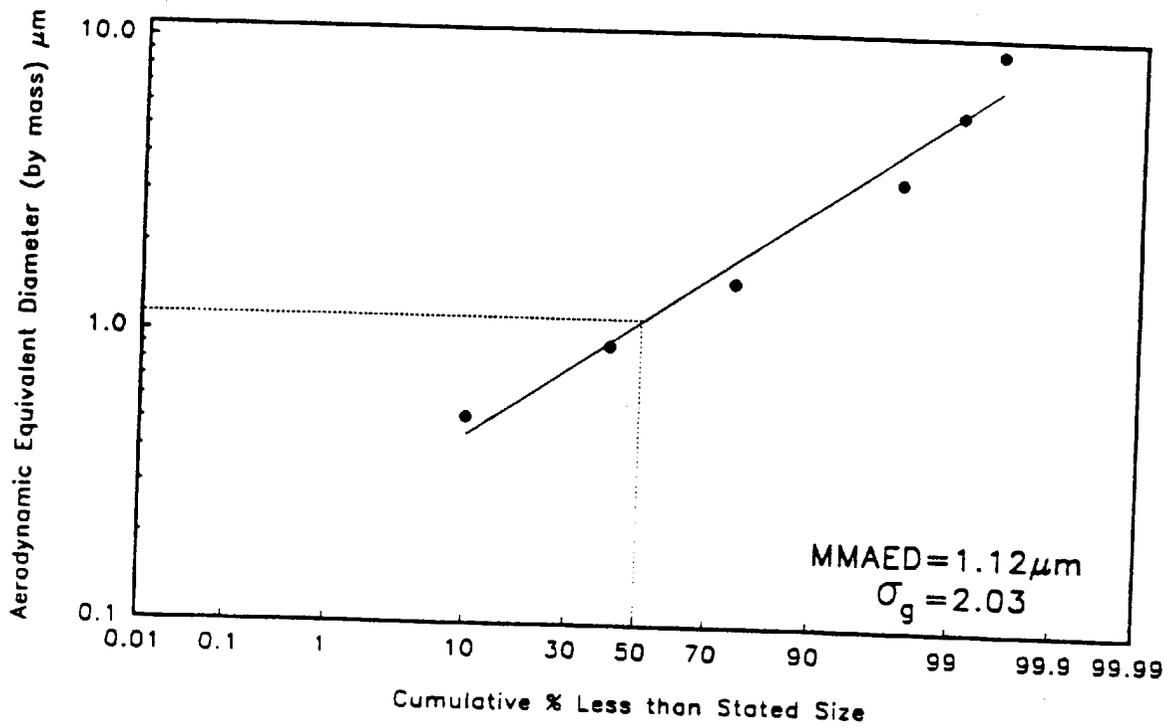
BORAMIDE
Particle Sizing



PETROLEUM OIL
 Concentration-Response relationships used to calculate RD_{50}
 including 95% C.I.

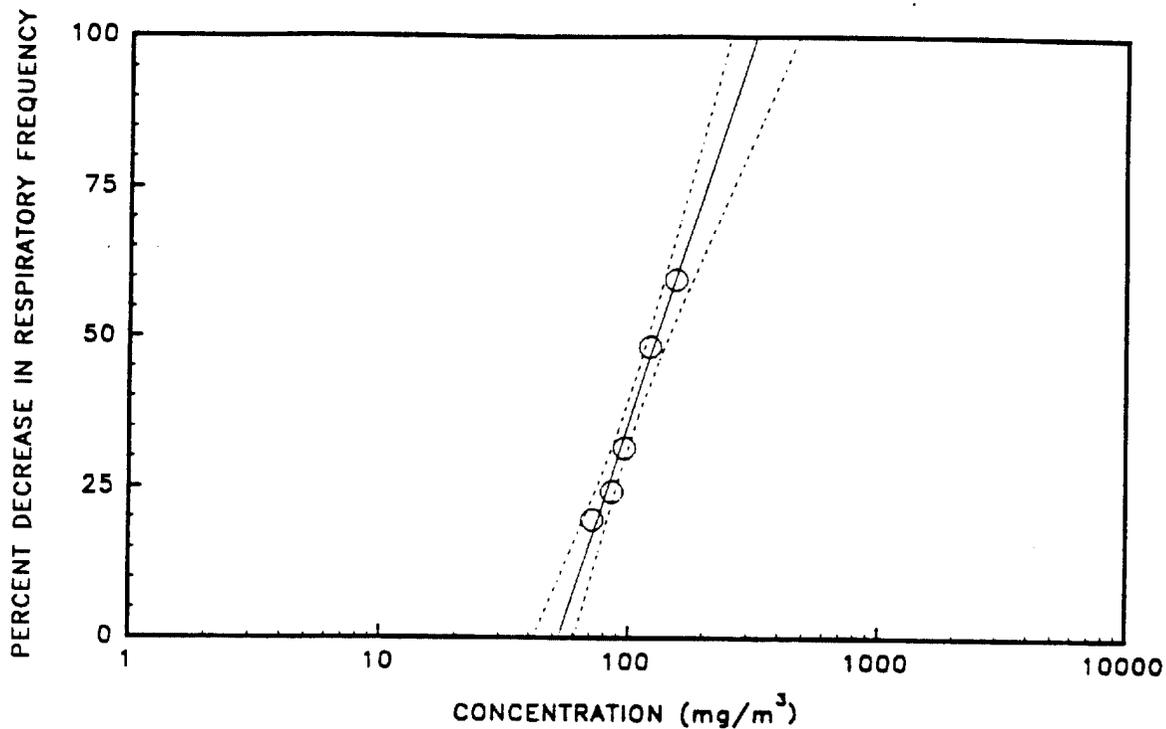


Particle Sizing

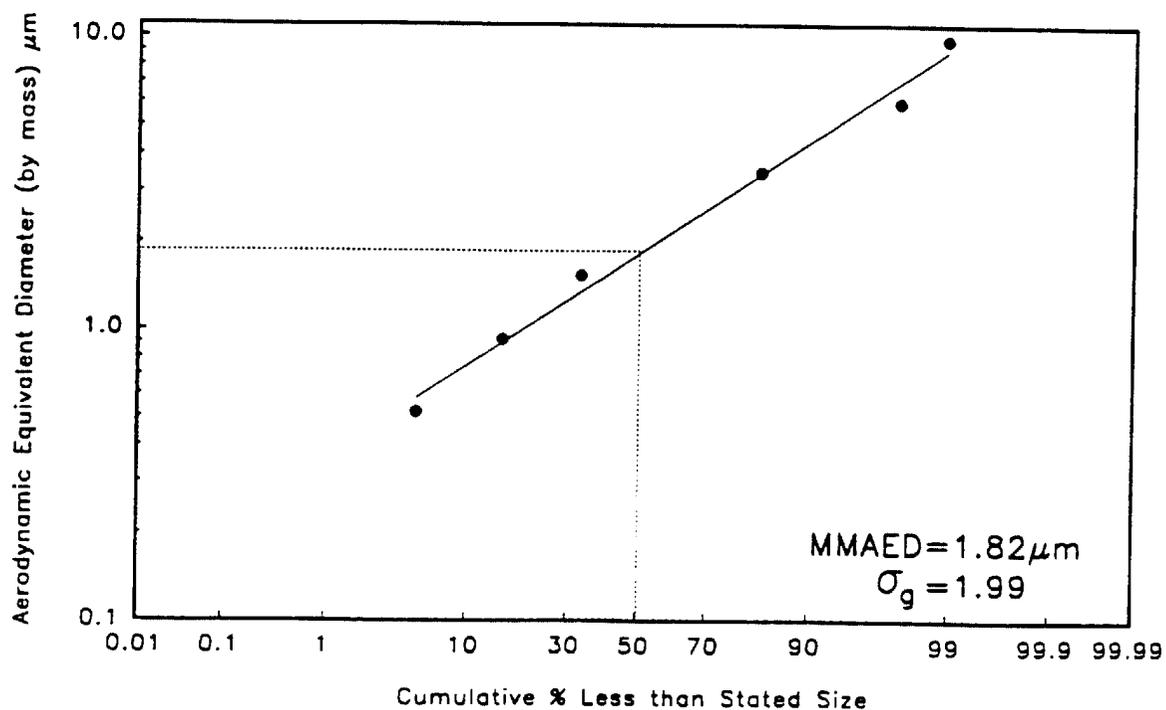


POTASSIUM SOAP

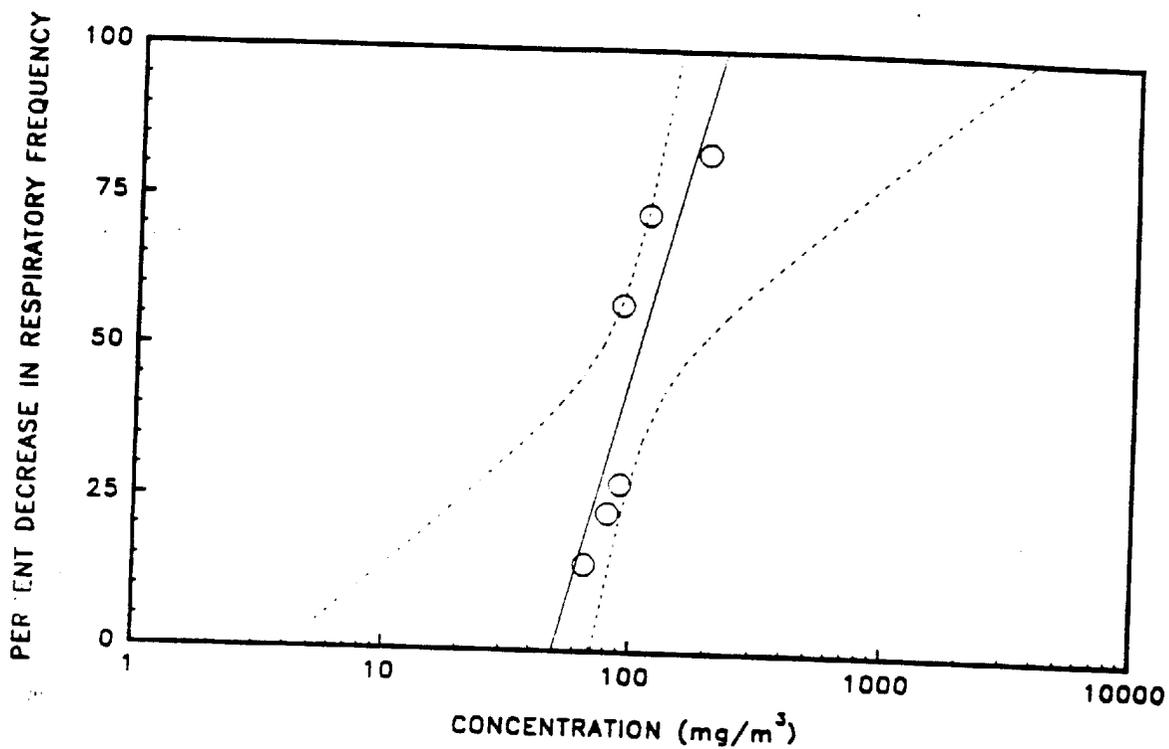
Concentration-Response relationships used to calculate RD_{50} including 95% C.I.



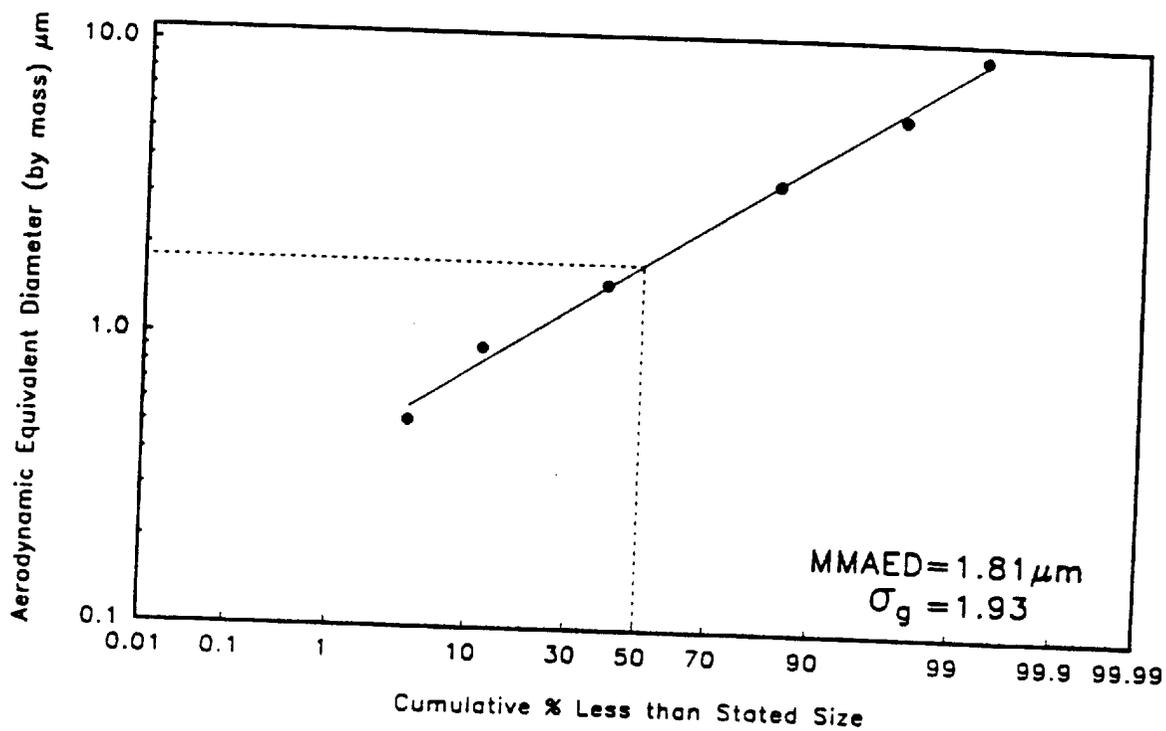
Particle Sizing



SODIUM SULFONATE
 Concentration-Response relationships used to calculate RD_{50}
 including 95% C.I.

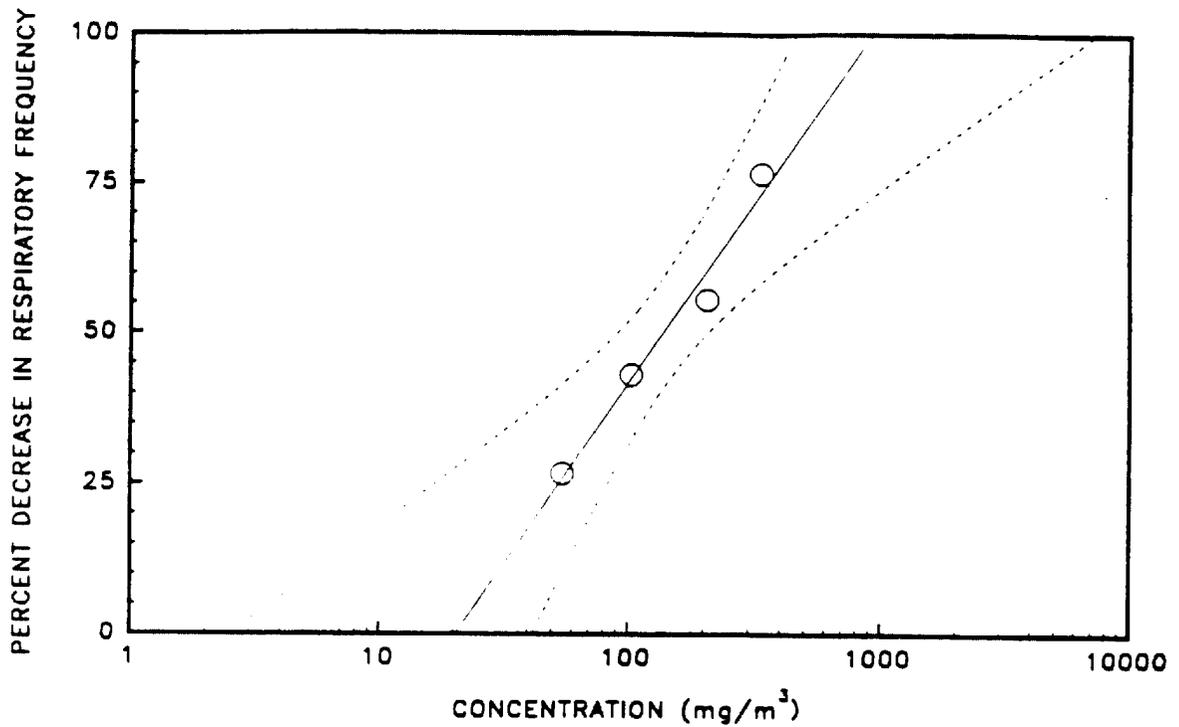
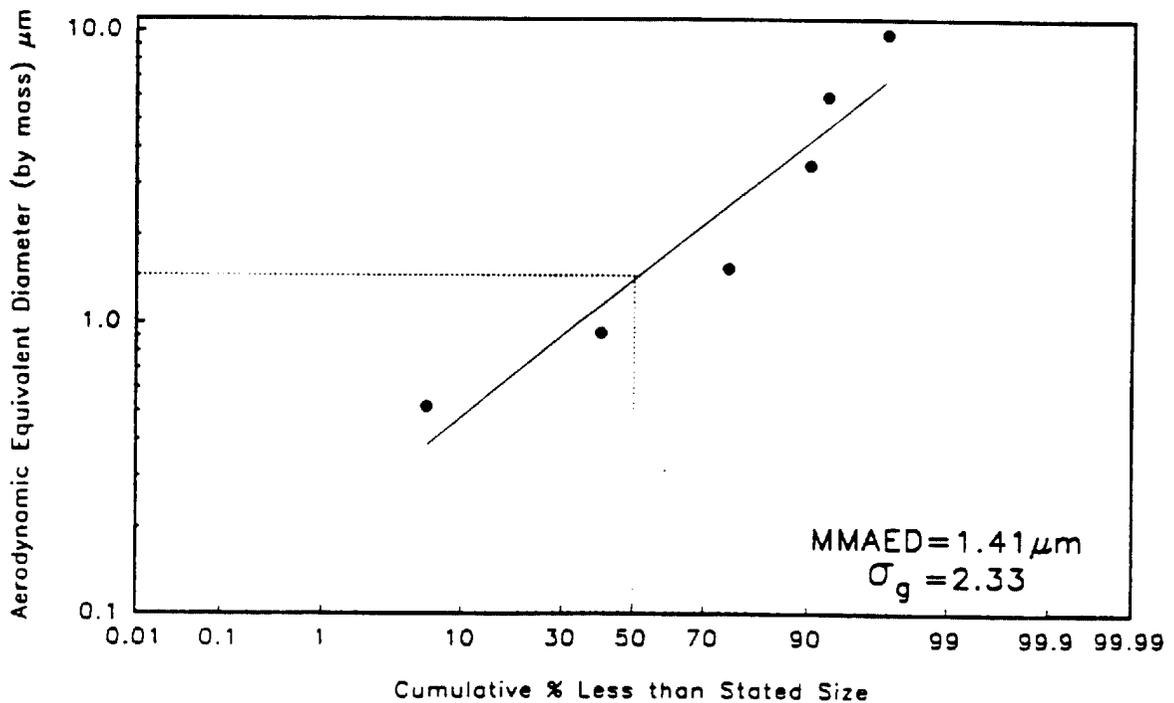


Particle Sizing

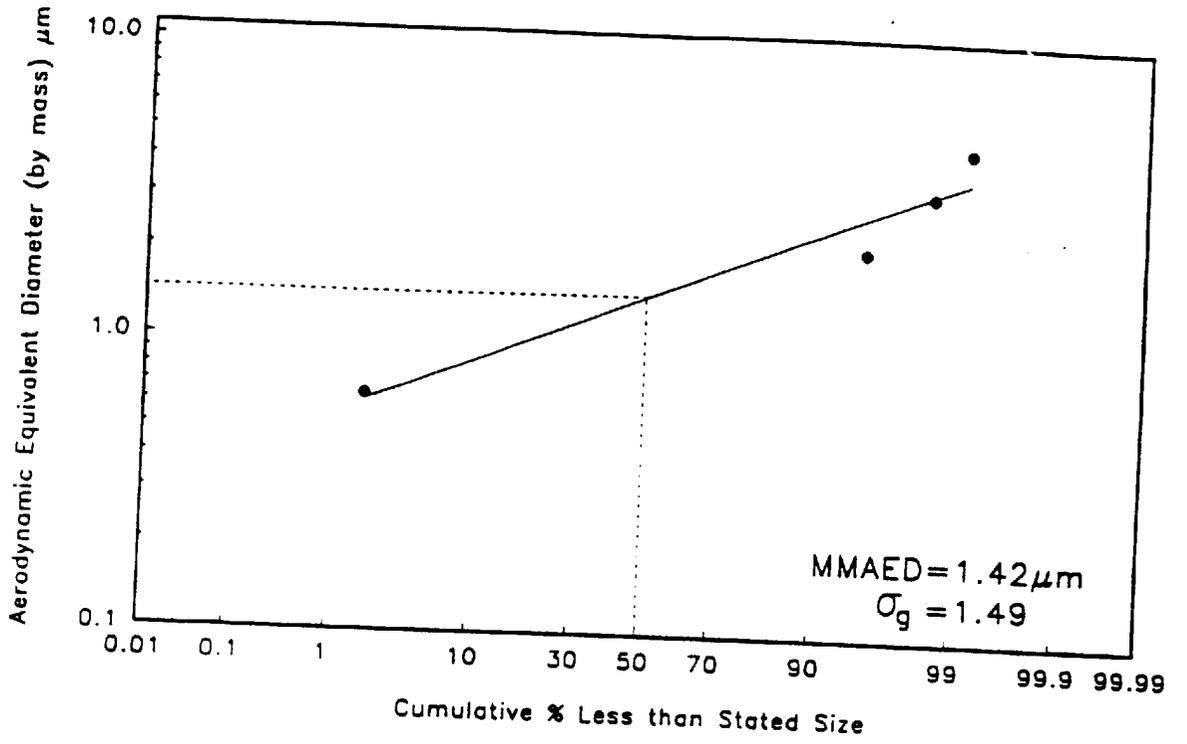


TRIAZINE

Concentration-Response relationships used to calculate RD_{50} including 95% C.I.

**Particle Sizing**

SAMPLE B
Particle Sizing



APPENDIX B CALCULATIONS

Sample calculation for nominal concentration determination:

Wt_0 = total weight of sample and generation equipment before generation of aerosol.

Wt_1 = total weight of sample and generation equipment plus collected overflow.

$Wt_0 - Wt_1$ = Weight lost during aerosol generation time period

T = Time of aerosol generation

E = Exhaust flow rate

T x E = Total amount of air exhausted during aerosol generation

$\frac{Wt_0 - Wt_1}{T \times E}$ = Nominal concentration in exposure chamber

Example- Alkanolamide #2:

Wt_0 = 1349.49 g

Wt_1 = 1349.233 g

$Wt_0 - Wt_1$ = .257 g = 257 mg

T = 70 min.

E = 20 lpm

T x E = 1400 l, 1000 l = 1 m³, T x E = 1.4 m³

257/1.4 m³ = 183.6 mg/m³

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OFFICE OF
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Sincerely,

Terry R. O'Bryan
Terry R. O'Bryan
Risk Analysis Branch

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13247 A



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NON-CAP

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Submission number: 13247A

TSCA Inventory:

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Study type (circle appropriate):

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Contractor reviewer: LPS Date: 1/20/95

CECATS DATA: 1194-13247 SEQ. A

Submission # BEHQ

INFORMATION REQUESTED: FLWP DATE:

0501 NO INFO REQUESTED
0502 INFO REQUESTED (TECH)
0503 INFO REQUESTED (VOL ACTIONS)
0504 INFO REQUESTED (REPORTING RATIONALE)
DISPOSITION:
0639 REFER TO CHEMICAL SCREENING
0678 CAP NOTICE

TYPE: INT SUPP FLWP

SUBMITTER NAME: General Motors Corporation

0601 NO ACTION REPORTED
0602 STUDIES PLANNED/IN PROGRESS
0603 NOTIFICATION OF WORK REPORTED
0604 LABELS/MSDS CHANGED
0605 PROCESS/ANALYSIS CHANGED
0606 APP USE DISCONTINUED
0607 PRODUCTION DISCONTINUED
0608 CONFIDENTIAL

SUB. DATE: 11/04/94 ORG DATE: 11/09/94 CSRAD DATE: 12/15/94

CHEMICAL NAME: Boric acid compound + 2,2',2''-triazine bionide

Amides, Telenil fatty, N,N-bis(hydroxyethyl) amide

Basic Acid

CASE # 8516-78-9 → 4719-04-4 → 10043-35-3

64742-52-5 Sulf 100 Texas oil

→ Boramide
68608-26-4 Sulfonic acids,
3077-30-3 petroleum,
68649-05-8 sodium salts
octanamide,
N,N-bis(hydroxyethyl) amide,
alcohol derivatives

INFO TYPE	PFC	INFO TYPE	PFC
0201 ONCO (HUMAN)	01 02 04	0216 EPICLIN	01 02 04
0202 ONCO (ANIMAL)	01 02 04	0217 HUMAN EXPOS (PROD CONTAM)	01 02 04
0203 CELL TRANS (IN VITRO)	01 02 04	0218 HUMAN EXPOS (ACCIDENTAL)	01 02 04
0204 MUTA (IN VITRO)	01 02 04	0219 HUMAN EXPOS (MONITORING)	01 02 04
0205 MUTA (IN VIVO)	01 02 04	0220 ECOAQUA TOX	01 02 04
0206 REPRO/TERATO (HUMAN)	01 02 04	0221 ENV. OCCUREL/FATE	01 02 04
0207 REPRO/TERATO (ANIMAL)	01 02 04	0222 EMER INCI OF ENV CONTAM	01 02 04
0208 NEURO (HUMAN)	01 02 04	0223 RESPONSE REOEST DELAY	01 02 04
0209 NEURO (ANIMAL)	01 02 04	0224 PRODCOMP/CHEM ID	01 02 04
0210 ACUTE TOX. (HUMAN)	01 02 04	0225 REPORTING RATIONALE	01 02 04
0211 CHR. TOX. (HUMAN)	01 02 04	0226 CONFIDENTIAL	01 02 04
0212 ACUTE TOX. (ANIMAL)	01 02 04	0227 ALLERG (HUMAN)	01 02 04
0213 SUB ACUTE TOX (ANIMAL)	01 02 04	0228 ALLERG (ANIMAL)	01 02 04
0214 SUB CHRONIC TOX (ANIMAL)	01 02 04	0229 METAB/PHARMACO (ANIMAL)	01 02 04
0215 CHRONIC TOX (ANIMAL)	01 02 04	0240 METAB/PHARMACO (HUMAN)	01 02 04

TRIAGE DATA: NON-CBI INVENTORY

ONGOING REVIEW

SPECIES

TOXICOLOGICAL CONCERN:

USE:

PRODUCTION:

YES

YES (DROPPED/REFER)

MOS

LOW

metal working fluid - lubricant

CAS SR NO

NO (CONTINUE)

MED

11/11/94

8111

HIGH

2 2 2 2 2

-CPSS- 1212952228

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> <ID NUMBER>

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> <TOX CONCERN>

L

> <COMMENT>

ALKANOLAMIDE 1: ACUTE INHALATION TOXICITY IN THE MOUSE IS OF LOW CONCERN. HEAD-ONLY EXPOSURE LEVELS (180-MINUTE) RANGING FROM 86 TO 219 MG/M3 PRODUCED BOTH PULMONARY AND SENSORY IRRITATION THROUGHOUT THE PERIOD OF EXPOSURE, ALTHOUGH ADVERSE EFFECTS WERE DELAYED AT LOWER CONCENTRATIONS. NO DEATHS WERE NOTED. SEVERITY OF RESPONSE WAS VARIABLE AMONG TEST ANIMALS OF AN EXPOSURE LEVEL. RECOVERY FOLLOWING EXPOSURE WAS POOR, ALTHOUGH ALL TEST ANIMALS SURVIVED AND, ON GROSS INSPECTION, APPEARED NORMAL AFTER 24 HOURS.

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> <ID NUMBER>

8(E)-13247A-02

> <TOX CONCERN>

L

> <COMMENT>

BORAMIDE: NEUROLOGICAL INHALATION TOXICITY IN THE MOUSE IS OF LOW CONCERN. THE MAXIMUM OBTAINABLE 180-MINUTE EXPOSURE (279 MG/M3) PRODUCED NO RESPIRATORY ANOMALIES INDICATIVE OF SENSORY OR PULMONARY IRRITATION.

BORIC ACID: NEUROLOGICAL INHALATION TOXICITY IN THE MOUSE BIOASSAY IS OF LOW CONCERN. THE MAXIMUM OBTAINABLE HEAD-ONLY 180 MINUTE EXPOSURE LEVEL (268 MG/M3) PRODUCED MINIMAL SENSORY AND PULMONARY IRRITATION ONLY AFTER 2 HOUR EXPOSURE. TEST ANIMALS' EYES WERE ENCRUSTED WITH BORIC ACID CRYSTALS FOLLOWING EXPOSURE.

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> <ID NUMBER>

8(E)-13247A-03

> <TOX CONCERN>

L

> <COMMENT>

BORIC ACID: NEUROLOGICAL INHALATION TOXICITY IN THE MOUSE BIOASSAY IS OF LOW CONCERN. THE MAXIMUM OBTAINABLE HEAD-ONLY 180 MINUTE EXPOSURE LEVEL (268 MG/M3) PRODUCED MINIMAL SENSORY AND PULMONARY IRRITATION ONLY AFTER 2-HOUR EXPOSURE. TEST ANIMALS' EYES WERE ENCRUSTED WITH BORIC ACID CRYSTALS FOLLOWING EXPOSURE.

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> <ID NUMBER>
8(E)-13247A-04

> <TOX CONCERN>
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> <COMMENT>
PETROLEUM OIL: ACUTE INHALATION TOXICITY IN THE MOUSE IS OF LOW CONCERN. EXPOSURE CONCENTRATIONS OF 1600 TO 3114 MG/M3 PRODUCED BOTH SENSORY AND PULMONARY IRRITATION EARLY WHICH LASTED THROUGHOUT THE 180-MINUTE DURATION OF HEAD-ONLY EXPOSURE. NO DEATHS WERE NOTED. RECOVERY FOLLOWING EXPOSURE WAS RELATIVELY RAPID. TEST ANIMALS WERE NOTED TO HAVE SWOLLEN HEADS AND OILY FUR, GROOMING THEMSELVES FOLLOWING EXPOSURE.

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> <ID NUMBER>
8(E)-13247A-05

> <TOX CONCERN>
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> <COMMENT>
SODIUM SULFONATE: ACUTE INHALATION TOXICITY IN THE MOUSE IS OF LOW CONCERN. NO DEATHS WERE NOTED. AT HIGHER EXPOSURE LEVELS, RESPIRATION RATES WERE DEPRESSED EARLY AND, OFTEN, CONTINUED TO FALL WITHOUT REACHING A PLATEAU AFTER 4-HOUR EXPOSURE. PULMONARY IRRITATION WAS SEVERE AT HIGHER CONCENTRATIONS (GREATER THAN 80 MG/M3). OVERT CLINICAL OBSERVATIONS INCLUDED IRRITATED AND "GLAZED" EYES, WET AND RUMPLED-APPEARING FUR THAT LASTED FOR 48 HOURS POST-EXPOSURE. AFTER 24 HOURS, MOST ANIMALS HAD LOST WEIGHT, UP TO 2 G. BY DAY 2 OR 3, RESPIRATORY PATTERNS WERE RELATIVELY NORMAL; BY DAY 3 ALL ANIMALS APPEARED RECOVERED.

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> <ID NUMBER>
8(E)-13247A-06

> <TOX CONCERN>
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> <COMMENT>
ALKANOLAMIDE 2: ACUTE INHALATION TOXICITY IN THE MOUSE BIOASSAY IS OF LOW CONCERN. EXPOSURE LEVELS (180-MINUTE), RANGING FROM 109 TO 302 MG/M3, PRODUCED IMMEDIATE PULMONARY AND SENSORY IRRITATION WHICH LASTED THROUGHOUT THE PERIOD OF EXPOSURE. NO DEATHS WERE NOTED. SEVERITY OF RESPONSE WAS VARIABLE AMONG TEST ANIMALS OF AN EXPOSURE LEVEL. THE ONLY OVERT CLINICAL MANIFESTATION WAS A CRYSTALLINE CRUST AROUND THE EYES OF ALL EXPOSED ANIMALS. BY DAY 2 (24 HOURS POST-EXPOSURE), ALL ANIMALS APPEARED NORMAL WITH RETURN

OF NORMAL RESPIRATORY PATTERNS AND FREQUENCIES.

INHALATION TOXICITY IN THE MOUSE IS OF LOW CONCERN. ALL HEAD-ONLY 180 MINUTE EXPOSURE LEVELS (109 TO 219 MG/M3) PRODUCED PULMONARY AND SENSORY IRRITATION. RD50 WAS 197 MG/M3. RECOVERY WAS POOR AT THE HIGHEST EXPOSURE LEVELS. THE ONLY OVERT CLINICAL SIGN WAS A CRYSTALLINE CRUST AROUND THE EYES OF ALL EXPOSED ANIMALS.

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> <ID NUMBER>
8(E)-13247A-07

> <TOX CONCERN>
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> <COMMENT>
TRIAZINE: ACUTE INHALATION TOXICITY IN THE MOUSE IS OF HIGH CONCERN BASED ON MORTALITY. HEAD-ONLY EXPOSURE LEVELS (180-MINUTE) AND ASSOCIATED MORTALITIES WERE AS FOLLOWS: 55 MG/M3 (1/3), 103 MG/M3 (1/4), 204 MG/M3 (2/4), 332 MG/M3 (2/4) AND 1619 MG/M3 (2/3). EXPOSURE-RELATED DEATHS WERE DELAYED, THE FIRST AT DAY 2 OF POST-EXPOSURE OBSERVATION. SENSORY AND PULMONARY IRRITATION AT THE HIGHER LEVELS OF EXPOSURE WERE IMMEDIATE, PROFOUND AND SUSTAINED AS INDICATED BY 24-HOUR OBSERVATION OF RESPIRATORY TIDAL VOLUME AND FREQUENCY DURING RECOVERY. WEIGHT LOSS AS HIGH AS 15% OCCURRED AMONG ANIMALS EXPOSED TO 103 MG/M3. TEST ANIMALS AT ALL EXPOSURE LEVELS BREATHED THROUGH THEIR MOUTHS, AND WERE NOTED TO MAKE CLICKING AND "CHIRPING SOUNDS". OVERT CLINICAL OBSERVATIONS INCLUDED SWOLLEN HEADS AND BACK LEG PARALYSIS DUE TO NECK DAMAGE, THE LATTER ATTRIBUTED TO ATTEMPTS AT EXTRICATION FROM TAPE COLLARS HOLDING ANIMALS' HEADS IN THE CHAMBER.

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> <ID NUMBER>
8(E)-13247A-08

> <TOX CONCERN>
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> <COMMENT>
POTASSIUM SOAP: ACUTE INHALATION TOXICITY IN THE MOUSE BIOASSAY IS OF LOW CONCERN. NO DEATHS WERE NOTED. SENSORY IRRITATION WAS NOTED EARLY AND LASTED THROUGHOUT THE PERIOD OF EXPOSURE (180 MINUTES); PULMONARY IRRITATION WAS MINIMAL. EXCEPT AT HIGHER EXPOSURE LEVELS, RECOVERY WAS RELATIVELY RAPID. OVERT CLINICAL OBSERVATIONS INCLUDED SWOLLEN HEADS AND IRRITATED EYES IN SOME TEST ANIMALS AND DAMP, SOAPY-APPEARING FACIAL FUR AT HIGHER EXPOSURE LEVELS. BY DAY 2 (24 HOURS) TEST ANIMALS' RESPIRATION WAS NORMALIZED.

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> <ID NUMBER>

8(E)-13247A-09

> <TOX CONCERN>
L/H

> <COMMENT>

TRIAZINE: ACUTE INHALATION TOXICITY IN THE MOUSE IS OF HIGH CONCERN BASED ON MORTALITY. HEAD-ONLY EXPOSURE LEVELS (180-MINUTE) AND ASSOCIATED MORTALITIES WERE AS FOLLOWS: 55 MG/M3 (1/3), 103 MG/M3 (1/4), 204 MG/M3 (2/4), 332 MG/M3 (2/4) AND 1619 MG/M3 (2/3). EXPOSURE-RELATED DEATHS WERE DELAYED, THE FIRST AT DAY 2 OF POST-EXPOSURE OBSERVATION. SENSORY AND PULMONARY IRRITATION AT THE HIGHER LEVELS OF EXPOSURE WERE IMMEDIATE, PROFOUND AND SUSTAINED AS INDICATED BY 24-HOUR OBSERVATION OF RESPIRATORY TIDAL VOLUME AND FREQUENCY DURING RECOVERY. WEIGHT LOSS AS HIGH AS 15% OCCURRED AMONG ANIMALS EXPOSED TO 103 MG/M3. TEST ANIMALS AT ALL EXPOSURE LEVELS BREATHED THROUGH THEIR MOUTHS, AND WERE NOTED TO MAKE CLICKING AND "CHIRPING SOUNDS". OVERT CLINICAL OBSERVATIONS INCLUDED SWOLLEN HEADS AND BACK LEG PARALYSIS DUE TO NECK DAMAGE, THE LATTER ATTRIBUTED TO ATTEMPTS AT EXTRICATION FROM TAPE COLLARS HOLDING ANIMALS' HEADS IN THE CHAMBER.

ALKANOLAMIDE 1: ACUTE INHALATION TOXICITY IN THE MOUSE IS OF LOW CONCERN. HEAD-ONLY EXPOSURE LEVELS (180-MINUTE) RANGING FROM 86 TO 219 MG/M3 PRODUCED BOTH PULMONARY AND SENSORY IRRITATION THROUGHOUT THE PERIOD OF EXPOSURE, ALTHOUGH ADVERSE EFFECTS WERE DELAYED AT LOWER CONCENTRATIONS. NO DEATHS WERE NOTED. SEVERITY OF RESPONSE WAS VARIABLE AMONG TEST ANIMALS OF AN EXPOSURE LEVEL. RECOVERY FOLLOWING EXPOSURE WAS POOR, ALTHOUGH ALL TEST ANIMALS SURVIVED AND, ON GROSS INSPECTION, APPEARED NORMAL AFTER 24 HOURS.

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BORIC ACID: NEUROLOGICAL INHALATION TOXICITY IN THE MOUSE BIOASSAY IS OF LOW CONCERN. THE MAXIMUM OBTAINABLE HEAD-ONLY 180 MINUTE

EXPOSURE LEVEL (268 MG/M3) PRODUCED MINIMAL SENSORY AND PULMONARY IRRITATION ONLY AFTER 2-HOUR EXPOSURE. TEST ANIMALS' EYES WERE ENCRUSTED WITH BORIC ACID CRYSTALS FOLLOWING EXPOSURE.

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