

# EPIDEMIOLOGY / TOXICOLOGY PLENARY SESSION

## Session Arranger / Moderator:

**NEIL C. HAWKINS, ScD, CIH**, Dow Chemical  
**JOHN K. HOWELL, PhD**, Castrol Industrial, North America  
**FRANKLIN E. MIRER, PhD, CIH**, United Auto Workers

## Discussants:

**LAWRENCE J. FINE, MD, DrPH**, Director Division of Surveillance, NIOSH  
**WILLIAM E. LUCKE, PhD**, Manager Regulatory Affairs, Cincinnati Milacron  
**LAWRENCE ROSLINSKI, PhD**, Manager, Toxicology Department, Ford Motor Company  
**MARGARET M. SEMINARIO**, Director Occupational Safety & Health, AFL-CIO

## Technical Presenters:

**JOHN R. BUCHER, PhD**, Chief, Toxicology Branch, National Institute of Environmental Health Sciences  
*NTP TOXICITY and CARCINOGENICITY STUDIES of METALWORKING FLUID COMPONENTS*

**WILLIAM T. STOTT, PhD**, Toxicology, Health & Environmental Sciences, Dow Chemical Company  
*ETHANOLAMINE TOXICITY*

**FREDERICK J. PASSMAN, PhD**, President, Biodeterioration Control Associates  
*BIOCIDE TOXICITY: A COMPARISON of the TOXICOLOGICAL PROPERTIES of COMMON METALWORKING FLUID BIOCIDES*

**MICHELLE M. SCHAPER, PhD**, Assoc. Professor of Environ. & Occup. Health, University of Pittsburgh  
*USE of a BIOASSAY to EVALUATE the RESPIRATORY IRRITANCY of METALWORKING FLUIDS and THEIR COMPONENTS*

**ANN M. BALL**, Biologist, Cincinnati Milacron  
*SENSORY IRRITATION POTENTIAL of COMMERCIALY AVAILABLE METALWORKING FLUIDS*

**PETER S. THORNE, PhD**, Assoc. Professor of Toxicology & Industrial Hygiene, University of Iowa  
*RESPIRATORY HEALTH EFFECTS of MACHINING FLUIDS in LABORATORY ANIMALS*

**DONALD K. MILTON, MD, DrPH**, Asst. Professor of Occup. Medicine, Harvard School of Public Health  
*ACUTE EFFECTS of METALWORKING FLUIDS in a RESPIRATORY INFLAMMATION MODEL*

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**FRANKLIN E. MIRER, PhD, CIH**, UAW

**Mr. DAVID FELINSKI, AAMA:** Good morning everyone, and welcome to day two of the Metalworking Fluids Symposium. Somehow, we ended up with no less than three Sessions Arrangers for this morning's session on Epidemiology and Toxicology, so it's unclear to me whether that was a function of the contentiousness or the complexity of the subject matter. At any rate, I have the privilege of introducing them to you, and in order to be politically correct, I shall introduce them in the order of who has the longest hair.

Franklin E. Mirer is a toxicologist and certified industrial hygienist. His primary scientific interest is exposure assessment in the occupational environment. Dr. Mirer serves as Director of the UAW Health and Safety Department. This technical unit coordinates the activities of the UAW in Occupational Health and Safety, and provides policy advice to the union's officers, assists with collective bargaining, conducts plant inspections, reviews technical and statistical data for all levels of the union, designs and delivers training programs, represents the union before administrative agencies and professional bodies, and conducts occupational safety and health research. Dr. Mirer has participated in each round of automobile industry collective bargaining since 1976.

Dr. Mirer received a Ph.D. in organic chemistry from Harvard University in 1972, and trained further as a Research Fellow in Toxicology at the Harvard School of Public Health. He joined the UAW staff in 1975 and was named Director of the Union's Health and Safety Department in 1982.

Dr. Mirer is a member of the Board of Scientific Counselors of the National Toxicology Program, the EPA Common Sense Initiative Advisory Council, and the Injury Advisory Committee to the Centers for Disease Control. He developed and delivered testimony before OSHA regarding a dozen health and safety standards, and has testified before House and Senate Committees on numerous occasions. He was a member of the National Academy of Sciences Committee on Institutional Means for Risk Assessment (which produced the 1983 "Red Book"), Committee on Risk Assessment Methodology, and Committee to Review the Health Effects Institute. Dr. Mirer has co-authored a number of scientific papers.

Neil Hawkins currently serves as Manager of Health Regulatory Compliance for the Dow Chemical Company. His group's responsibilities include TSCA, FDA-related issues, OSHA, FIFRA, and global coordination for related activities. Dr. Hawkins has previously been group leader for Chemicals Product Stewardship and served in risk assessment and industrial hygiene positions in Dow's Health and Environmental Sciences department.

Dr. Hawkins has published extensively on risk assessment and serves on the Editorial Board of the American Industrial Hygiene Association Journal. Within the AIHA, he is certified in the comprehensive practice of Industrial Hygiene, and is currently co-chair of the Risk Assessment Committee and past-chair of the Exposure Assessment Strategies Committee.

Dr. Hawkins studied Health Physics at Georgia Tech and later earned his Masters and Doctorate of Science Degrees in Environmental

Health Sciences from Harvard University, School of Public Health.

John Howell received his B.S. and Ph.D. degrees in Chemistry from Drexel University in Philadelphia, and later, his M.A. in Organization Development from Loyola University in Chicago. After spending ten years as a research chemist, group leader, and technical manager for Parker-Amchem, Dr. Howell joined Van Straaten Corporation in 1980 as Director of Research. After Castrol acquired Van Straaten in 1987, Dr. Howell held similar positions with the new Castrol Industrial organization. Today, Dr. Howell is Director, Health and Environmental Affairs for Castrol Industrial North America in Downers Grove, Illinois.

Dr. Howell has been active in the Independent Lubricant Manufacturers Association for several years and is current Chair of their Health and Safety Task Force. Dr. Howell is also active in the Society of Tribologists and Lubrication Engineers and has made several presentations at that group's national meetings.

Additionally, Dr. Howell is Chairman of the ASTM Subcommittee that writes Health & Safety Standards for Metalworking Fluids, which has developed three standards in this area over the last several years. Gentleman, the Session is yours.

**Dr. NEIL HAWKINS, Dow Chemical:** I want to welcome everyone to this morning's session. Yesterday, we had a session on the epidemiology of metalworking fluids, which focused primarily on mortality. Today we are moving into more of a toxicology session, that will evolve into another epidemiology session on respiratory effects later this afternoon. Our first speaker this morning is John Bucher. He is Chief of the Toxicology Branch of the National Institute of Environmental Health Sciences. His topic will be NTP Toxicity and Carcinogenicity Studies of Metalworking Fluid Components. Please welcome Dr. Bucher.

## National Toxicology Program Toxicity and Carcinogenicity Studies of Metalworking Fluid Components

John R. Bucher

National Institute of Environmental Health Sciences  
Research Triangle Park, NC 27709

### ABSTRACT

The National Toxicology Program (NTP) has studied a number of common components of metalworking or machining fluids for toxic and carcinogenic properties in rodents. Evaluations of genetic and/or reproductive and developmental toxicity, general toxicity and/or carcinogenicity were performed on ethanolamine, diethanolamine, triethanolamine, two chlorinated paraffins and dimethoxane. In terms of relative hazard of the chemicals studied, diethanolamine may pose the greatest potential for general toxicity, and a relevant mechanism involving incorporation into phospholipids has been identified. In terms of carcinogenic hazard, the short chain, highly chlorinated paraffin would appear to pose the greatest threat, although the viscous liquid physical characteristic may act to limit exposure. The NTP Seventh Annual Report on Carcinogens lists only chlorinated paraffins (C<sub>12</sub> 60% Cl) of the chemicals considered in this report, as a chemical "reasonably anticipated to be carcinogenic to human

### INTRODUCTION

The National Toxicology Program has performed several types of toxicology and rodent carcinogenesis studies on chemicals that are used in metal cutting or machining fluids. The chemicals were studied individually and were not considered as a class, thus routes of administration and study designs varied. Two chlorinated paraffins used as extreme pressure lubricants, a long chain moderately chlorinated form, and a shorter chain more highly chlorinated form, as well as dimethoxane, an antimicrobial agent in cutting fluids, were given by oral gavage in corn oil in pre-chronic and chronic rat and mouse toxicity and carcinogenicity studies.

Triethanolamine was studied by topical administration in acetone in toxicology and carcinogenesis studies and by oral corn oil gavage in rat teratology studies. Diethanolamine was studied by the topical and drinking water routes in 13-week rat and mouse toxicity studies and by oral corn oil gavage in rat teratology studies. Ethanolamine was also evaluated by corn oil gavage for teratogenic effects in rats.

In addition, these chemicals were evaluated in a battery of *in vitro* and *in vivo* tests for mutagenic or other genotoxic effects. Tests for mutagenicity, typically with and without metabolic activation, were performed using the Ames *Salmonella* test as well as a test for induction of trifluorothymidine resistance in mouse lymphoma L5178Y cells. Mutagenicity was also evaluated by the induction of sex-linked recessive lethal mutations in *Drosophila melanogaster* given the chemicals by injection or feeding. The induction of sister chromatid exchange and chromosomal aberrations was assessed *in vitro* studies using Chinese hamster ovary cells. The purpose of these collected studies was to identify potential hazards associated with the human use or exposure to these chemicals in consumer products or in occupational settings.

### METHODS and RESULTS

The materials and methods used in the prechronic and chronic rodent toxicity and carcinogenicity studies have been reported in various technical reports.<sup>(1-5)</sup> Briefly, 14 day, 13-week and 2-year studies were performed by exposing male and female F344/N rats and B6C3F1 mice to the chemicals continuously in drinking water, or once per day, five days per week by gavage or topical administration in the indicated vehicle. A variety of endpoints

including clinical signs, body weights, clinical pathology and others were monitored, and a complete histopathological evaluation was performed at the end of the study. Teratology studies were performed by exposing pregnant Sprague-Dawley rats to the chemicals by gavage during gestation days 6 to 15. C-sections were performed on the day prior to normal delivery and pups were scored for abnormalities. Space does not permit a description of the genetic toxicity evaluations.

In genetic toxicity studies, both chlorinated paraffins and dimethoxane were mutagenic in mouse lymphoma cells and caused chromosomal aberrations and/or sister chromatid

exchange in Chinese hamster ovary cells. Dimethoxane was also mutagenic in the *Salmonella* assay, as well as in *Drosophila*. In contrast, the ethanolamine compounds were uniformly negative in the genetic toxicology battery.<sup>(1-5)</sup>

The pre-chronic and chronic toxicity studies performed with these chemicals are indicated in Table 1. Some studies were done by one or more routes of administration. The results of all studies are reported except for the 2-year studies of diethanolamine, anticipated to be ready in June 1996, and the triethanolamine studies currently available only as a draft report.

Toxicity and Carcinogenicity Studies with Chlorinated Paraffins, Triethanolamine, Diethanolamine and Dimethoxane				
		14-Day	13-Week	2-Year
Chlorinated Paraffins	C <sub>23</sub> 43% Cl (gavage)	X	X	X
	C <sub>12</sub> 60% Cl (gavage)	X	X	X
Triethanolamine	(Topical)	X	X	X
	(Drinking water)	X	--	--
	(Inhalation)	X	--	--
Diethanolamine	(Topical)	X	X	June '96
	(Drinking water)	X	X	--
Dimethoxane	(Gavage)	X	X	X

Table 1

There were few signs of toxicity of chlorinated paraffin C<sub>23</sub> 43% Cl in rats or mice given even very large doses in prechronic studies. Only in the female rat was there evidence of granulomatous inflammation in the liver at doses of 300 mg/kg or higher. There was no *evidence of carcinogenicity* in male rats given doses as high as 3,750 mg/kg/day in the 2-year studies. There was *equivocal evidence of carcinogenicity* in female rats given doses up to 900 mg/kg/day based on a marginal increase in adrenal medullary pheochromocytomas. There was also *equivocal evidence* in female mice given doses of up to 5,000 mg/kg/day based on a slight increase in hepatocellular neoplasms, however malignant

lymphomas were significantly increased in male mice, leading to a call of *clear evidence of carcinogenicity*.<sup>(1,6)</sup>

The liver was a target organ in prechronic studies with the chlorinated paraffin C<sub>12</sub>, 60% Cl. The primary lesion in rats was a marked hepatocellular hypertrophy seen at doses of 1,250 mg/kg and higher in both males and females. In mice, the hypertrophy was accompanied by focal hepatocellular necrosis. These lesions occurred at doses of 250 ppm or higher in both sexes of mice. In the 2-year studies there was *clear evidence of carcinogenicity* in all 4 sex/species groups. Hepatocellular neoplasms were increased in all dosed groups, and thyroid follicular cell

neoplasms were increased in female rats and female mice. Male rats also had an increase in renal tubular cell adenomas.<sup>(2,6)</sup>

Pre-chronic toxicity studies of triethanolamine revealed a rather marked acanthosis and inflammation at the site of application of the triethanolamine/acetone solutions, and female rats showed increased nephrosis. These findings occurred with doses of about 250 mg/kg/day and above, but inflammation in the skin was much less severe in mice compared to rats, leading to higher doses for the 2-year mouse studies than were used with rats. In the two year studies, the incidence and size of renal tubular adenomas was marginally increased in male rats and this was considered *equivocal evidence of carcinogenicity*. In female rats there was *no evidence of carcinogenicity* in animals receiving doses of up to 250 mg/kg/day. Both male and female mice had increased incidences of hepatocellular neoplasms and this was considered *some evidence of carcinogenicity* in females. The study in male rats was complicated by evidence suggestive of an infection with *Helicobacter hepaticus*, a bacterium known to cause liver neoplasia in male, but not female mice. Nonetheless, the incidence of liver neoplasia in dosed males lacking histologic evidence of infection was marginally greater than that in similar controls, and this was deemed *equivocal evidence of carcinogenicity*.<sup>(5)</sup> The tissues of female mice are currently being evaluated for evidence of *Helicobacter* infection by the Alkanolamines Panel of the Chemical Manufacturers Association.

Diethanolamine has been evaluated in 13-week studies by the drinking water route, and by topical application in ethanol. The basic pattern of lesions was similar with both routes, but was more severe when given in the water. Mortality occurred at topical doses of 500 to 1,250 mg/kg and above and 5,000 and 2,500 ppm in drinking water given to rats and mice respectively. Lesions common to both routes included anemia and demyelination of the brain and/or spinal cord in rats, and spectrum of hepatocyte alterations in mice. Nephropathy and testicular degeneration

was also seen in the drinking water study in rats. Lesions at the site of dermal application also occurred in both species. Examination of phospholipids from diethanolamine-treated rats showed inappropriate incorporation of diethanolamine, presumably in place of ethanolamine, possibly accounting for the derangements in myelination.<sup>(4,7,8)</sup> Two year studies of diethanolamine carcinogenicity by topical application will be reported in June 1996.

Pre-chronic and 2-year studies of dimethoxane were performed by oral corn oil gavage. There was little toxicity seen in 13-week studies other than irritation and acanthosis of the forestomach in rats and mice, and weight gain depression in rats. In 2-year studies there was *no evidence of carcinogenicity* in male and female rats and female mice, given doses of up to 125, 250, or 500 mg/kg/day respectively. There was *equivocal evidence of carcinogenicity* in male mice based on a few forestomach neoplasms observed in dosed animals.<sup>(3)</sup>

Ethanolamine, diethanolamine and triethanolamine were evaluated for potential teratogenic effects by gavage administration to pregnant Sprague-Dawley rats. There was no evidence of teratogenic effects in any of the studies (NTP, unpublished).

## CONCLUSIONS

In terms of relative hazard, it would appear that of the chemicals studied, diethanolamine may pose the greatest potential for general toxicity, and a relevant mechanism has been identified. In terms of carcinogenic hazard, the short chain, highly chlorinated paraffin would appear to pose the greatest threat, although the physical characteristics would tend to suggest a rather limited exposure potential. The NTP Seventh Annual Report on Carcinogens lists only chlorinated paraffins (C<sub>12</sub> 60% Cl) of the chemicals considered in this report, among those chemicals "reasonably anticipated to be carcinogenic to humans."

## REFERENCES

1. NTP (1986) Technical Report on the

- Toxicology and Carcinogenesis Studies of Chlorinated Paraffins (C<sub>23</sub> Cl 43%) In F344/N Rats and B6C3FI Mice, NIH Publication 86-2561, U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. 202 p.
2. **NTP** (1986a) Technical Report on the Toxicology and Carcinogenesis Studies of Chlorinated Paraffins (C<sub>12</sub> Cl 58%) In F344/N Rats and B6C3FI Mice, NIH Publication 86-2564, U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. 206 p.
  3. **NTP** (1989) Technical Report on the Toxicology and Carcinogenesis Studies of Dimethoxane in F344/N Rats and B6C3FI Mice, NIH Publication 89-2809, U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. 206 p.
  4. **NTP** (1992) Technical Report on Toxicity Studies of Diethanolamine Administered Topically and in Drinking Water to F344/N Rats and B6C3FI Mice. NIH Publication 923343, U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. 68 p.
  5. **NTP** (1994) Draft Technical Report on the Toxicity and Carcinogenicity of Triethanolamine in F344/N rats and B6C3FI mice, NIH Publication 94-3365, U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, 102 p.
  6. **Bucher, JR; R Alison, CA Montgomery, J Huff, JK Haseman, D Farnell, R Thompson, and JD Prejean:** (1987) Comparative toxicity and carcinogenicity studies of chlorinated paraffins in F344/N rats and B6C3F I mice. *Fund. Appl. Toxicol.* 9:454-468.
  7. **Melnick, RL; J Mahler, JR Bucher, M Thompson, M Hejtmancik, MJ Ryan, and LE Mezza:** (1994) Toxicity of diethanolamine. 1. Topical application and drinking water exposures in F344 rats. *J. Appl. Toxicol.* 14:1-9.
  8. **Melnick, RL; J Mahler, JR Bucher, M Thompson, M, Hejtmancik, A Singer, and RL Persing:** (1994) Toxicity of diethanolamine. 2. Topical application and drinking water exposures in B6C3FI mice. *J. Appl. Toxicol.* 14:11-19.

## Alkanolamine Toxicity

William T. Stott

Dow Chemical Company, Midland, MI 48674

### ABSTRACT

Occupational exposure to mono-, di-, and/or tri-ethanolamine (MEA, DEA and TEA, respectively) may occur during the use of metal working fluids containing these compounds. A number of studies have been undertaken over the last 45 years to characterize the potential dermal, oral and/or inhalation toxicities of MEA, DEA, and TEA. In general, ethanolamines are of low acute oral toxicity, may cause skin irritation at relatively high concentrations and are not sensitizers in controlled laboratory tests. Upon more prolonged dosing, the liver and/or kidneys are typically identified as target tissues as well as the skin in dermal studies. A number of other target tissues have been identified for DEA, apparently due to its misincorporation into cell membrane lipids. Despite this, ethanolamines are negative in teratogenicity assays indicative of a lack of potential to cause birth defects. These compounds also lack genotoxicity indicating a general lack of intrinsic potential to cause cancer in animals. Finally, a single ethanolamine, TEA, has also been extensively evaluated for oncogenic potential in laboratory animals under a variety of study designs. There have been a few reports of relative increases in specific tumor types in animals chronically administered high dosages of TEA, however, in each case a number of potentially confounding factors have resulted in uncertainties regarding the accuracy of the findings and/or their relevance in terms of human risk assessment. The findings of several bioassays are discussed in some detail. It is concluded that under appropriate usage, ethanolamines do not pose a risk to human health.

### General Toxicity of Ethanolamines

Ethanolamines, especially DEA and TEA, typically make up approximately 3% or less of synthetic and semi-synthetic metal working fluids.

Thus, exposure of workers to ethanolamines may occur via dermal contact or inhalation of respirable aerosols during use of metal working fluids under a number of occupational settings. The potential toxicities of MEA, DEA and TEA have been examined in a number of studies conducted over the last 45 years. Dependent on the particular ethanolamine, a broad spectrum of toxicological evaluations have been undertaken involving a variety of experimental models, dosing routes and durations, and study designs. The potential acute toxicity, sensitization potential, repeated dose toxicity, teratogenicity, oncogenicity, genotoxicity, metabolism, and ecotoxicity have been investigated. Extensive reviews of these studies have been reported by Benya and Harbison,<sup>(1)</sup> BIBRA,<sup>(2-4)</sup> CTFA,<sup>(5)</sup> and Knaak *et al.* (manuscript in preparation).

Ethanolamines, in general, have relatively low acute oral toxicity, can be irritating and, dependent upon chain length, even corrosive to skin at high concentrations. In addition, these compounds are not sensitizers under controlled experimental conditions. More prolonged, repeated dosing of experimental animals via oral, inhalation, and/or dermal routes of administration have revealed some common target tissues. These have been the liver and/or kidneys for all three compounds and erythrocytes and central nervous system tissues for DEA. Treatment-related changes, both adaptive and pathologic, in these tissues are consistent with the apparent metabolism by hepatic tissues, excretion primarily via the urine and, in the case of DEA, misincorporation into the phospholipids of cell membranes of a variety of tissues. Skin effects noted in animals administered ethanolamines via dermal application or, in some cases, whole body inhalation exposure, reflect the potential irritant properties of these strongly alkaline materials, especially at the high concentrations often utilized

in toxicity testing. A number of additional histopathologic changes have also been reported in animals administered relatively high dosages of ethanolamines; however, the significance of these changes have been difficult to ascertain due to confounding factors such as increased mortality, severe body weight depression, and stress-related changes.

The administration of relatively high, maternally toxic dosages of ethanolamines have not reproducibly produced birth defects (terata) in experimental animals. A reported positive finding in a single oral dosing rat study involving an unusual study design was not reproducible.

As a chemical family, ethanolamines lack genotoxic activity and thus are unlikely to pose a carcinogenic hazard to animals at dosages below those causing chronic tissue damage. A potential exception to this is the reaction of the secondary amine, DEA, with nitrite under favorable chemical conditions to form the mutagenic and carcinogenic nitrosamine N-nitrosodiethanol-amine (NDELA). A similar reaction has been suggested to occur for the tertiary amine, TEA; however, this reaction would appear to occur so slowly as to not be a factor in the toxicity of this latter compound.

A considerable amount of data has also been generated upon the potential oncogenicity of one particular ethanolamine, TEA, under a variety of study designs. The results of these bioassays have been somewhat contradictory and occasionally controversial but, in general, have failed to demonstrate clear evidence of a carcinogenic response for this compound. Given the extensive use of this latter ethanolamine in metal working fluids, a closer examination of several of these bioassays is undertaken below.

### **Chronic Toxicity and Oncogenic Testing of Triethanolamine**

A total of 5 chronic toxicity/oncogenicity studies of varying quality have been conducted in which TEA has been administered to test animals via the oral or dermal routes of exposure. A striking feature of all these studies has been the relatively high dosages tolerated by test animals, especially mice, for prolonged periods of time,

despite the known irritant properties of concentrated TEA solutions. As with other ethanolamines, the kidneys and livers of test species have typically been observed to be the most sensitive systemic target tissues of TEA while the skin represents a target tissue in dermal toxicity studies. Consistent with the lack of genotoxicity of TEA in standard assays, no clear-cut indication that this chemical may cause cancer in test animals has been found. As noted, there have been a few reports of relative increases in specific tumor types in animals chronically administered high dosages of TEA, but in each case, a number of potentially confounding factors have left questions regarding the accuracy of the findings and/or their relevance in terms of human risk assessment.

**Oral Chronic/Oncogenicity Bioassays.** The potential chronic toxicity and oncogenicity of orally administered TEA has been evaluated in both rats and mice. Maekawa *et al.*<sup>(6)</sup> provided male and female Fischer 344 rats drinking water containing 0 (control), 1%, or 2% of a high purity TEA for two years followed by tap water for an additional 9 weeks. The dose levels in females were reduced by half from week 69, because of excessive treatment-related nephrotoxicity. Based upon reported body weight and water consumption data, dosages were approximately 525 and 1100 mg/kg/day in males and, initially, approximately 910 and 1970 mg/kg/day in females. Subsequent to week 69 of the dosing period, dosages in females were approximately 455 and 985 mg/kg/day. Total TEA intake over the two-year period was calculated to be 170 and 119 grams per rat in males and females, respectively, imbibing the 1% TEA solution, and 358 and 232 grams per rat in males and females, respectively, imbibing the 2% solution.

The most significant treatment-related effects observed by Maekawa *et al.* were depressed body weights and changes in kidneys. Terminal body weights of treated males and females were decreased as much as 10% and 14%, relative to controls, respectively, while kidney weights of male and female rats were increased. Microscopic

changes consisted of an "acceleration of so-called chronic nephropathy" commonly observed in the kidneys of aging Fischer 344 rats. In addition, kidney tissues of these animals had a greater degree of mineralization, cell proliferation (hyperplasia) and inflammation with or without cell death (necrosis) than controls. Positive trends in the occurrence of liver tumors in males and of uterine and benign kidney tumors in females were attributed to the unusually low incidence of these tumors in controls relative to laboratory historical values. Further, the occurrence of renal tumors in high dose group female rats were attributed to the extensive kidney toxicity observed in these animals. The roughly 14% decreased body weights and increased mortality of high dose group females relative to controls (42% vs. 16%), indicates that the maximum tolerated dosage (MTD) was exceeded. The authors concluded that TEA was not carcinogenic.

The chronic oral toxicity and oncogenicity of TEA has also been evaluated in mice. Male and female B6C3F1 mice were provided drinking water containing 0, 1.0 or 2.0% TEA (approximately 98% purity with 1.9% DEA) for approximately 82 weeks.<sup>(7)</sup> Total dosages of TEA administered in the study by mice imbibing 1% and 2% solutions were calculated to be approximately 27-37 g/mouse and 63-64 g/mouse. It is estimated that maximum dosages averaged over 3,000 mg/kg/day TEA in both sexes of high dose group mice. Average body weights of all groups of animals were similar during the dosing period with the exception of high dose group males and females which were depressed during the last 3-4 months of dosing. Despite this, no treatment-related changes were associated with TEA imbibition. The authors concluded that the results of the study "documented a lack of carcinogenic activity of triethanolamine in B6C3F1 mice."

One additional oral bioassay has been reported for TEA by Hoshino and Tanooka.<sup>(8)</sup> This study, however, has been highly criticized for a number of design and data interpretation shortcomings and its findings found to be of no value for risk assessment.

#### **Dermal Chronic/Oncogenicity Bioassays.**

Several bioassays utilizing dermal administration of TEA have also been conducted in rats and mice. There is some question regarding the actual route of administration in these studies as TEA was simply "painted" on the backs of test animals, providing the opportunity for animals to actually ingest a significant portion of the dosage during grooming. In addition, the potential impact of various methodological, interpretive and even disease problems upon the results of these studies suggest that caution should be exercised in the use of these data for risk assessment.

In a recently released rat bioassay, varying amounts of an acetone solution of TEA (55-272  $\mu$ l/rat) were "painted" on the shaved backs of male and female Fischer 344 rats at dosages of 0, 32, 64 or 125 mg/kg/day and 0, 63, 125 or 250 mg/kg/day, respectively, 5 days/week, for 2 years.<sup>(9,10)</sup> Based upon mean body weight data, the total estimated lifetime dosages of TEA administered high dose group males and females were approximately 24 and 31 grams per rat, respectively. These dosages were roughly 10-fold lower than dosages administered to rats by Maekawa *et al.* in an oral bioassay of TEA. Vehicle control groups of rats were administered acetone only; however, no untreated controls were included in the study. It is thus difficult to ascertain the potential effects of the acetone vehicle upon the results of the study, for example via acetone's effects upon the absorption and skin irritancy of TEA. The survival rate of high dose group females to study termination was less than that of controls, 36% vs. 50%. The body weights of these same animals were also depressed by as much as 12% relative to controls during the dosing period and roughly 7% by terminal sacrifice. Not surprisingly, a number of changes indicative of chronic irritation were observed in skin at the site of application in both sexes of treated rats, including ulcerations and erosions.

Treatment-related effects were primarily limited to kidney tissues. Kidneys were examined using both the "standard" microscopic evaluation and an additional extensive "step-section" evaluation. Kidney weights of high dose group females were elevated, however, no dose-related

increase in microscopic changes were noted in renal tissues of these or any other rats. Chronic, "old-age" kidney disease, typically seen in this strain of rat, was observed to a similar degree in controls and treated animals alike. Microscopic examination of renal tissues also revealed a number of animals with hyperplasia of the tubular epithelium and relatively small, microscopic, benign tumors (adenomas). The incidence of renal adenomas (8%) observed during the "standard" evaluation of tissues in males administered the intermediate dosage of 63 mg/kg/day TEA was numerically higher than in controls and slightly higher than the highest incidence reported in untreated historical controls (0-6%). Interestingly, evaluation of step-sectioned kidneys, with its significantly increased statistical power to detect lesions relative to standard sectioning procedures, failed to identify a greater number of tumors in treated rats than the standard method. Upon combining the results of the two evaluations, the total yield of adenomas lacked good dose-response, being 2, 4, 12, and 8% in ascending dose level. Results of the study are further confounded by the fact that a review of the study revealed that the distinction between hyperplasia, a potential precursor of adenomas, and the very small adenomas themselves observed, was relatively subtle. Combining hyperplasia and adenomas together to get a measure of the occurrence of total "proliferative lesions" resulted in an almost identical incidence between control and treatment groups of rats (20-26%). These latter data suggest a lack of a tumorigenic response in the kidneys of male rats. No treatment-related increase in tumor incidence was noted in any other organ system in male rats nor in any treatment groups of female rats. It was concluded that overall the study failed to generate clear evidence of a carcinogenic response in rats and that the male kidney tumor data was "equivocal."

In a companion study, the potential chronic toxicity and oncogenicity of dermally administered TEA was evaluated in B6C3F1 mice.<sup>(9,10)</sup> As in the rats, varying amounts of an acetone solution of TEA (33-105 l/mouse) were applied to the shaved backs of male and female B6C3F1 mice at dosages

of 0, 200, 1000 or 2000 mg/kg/day, and 0, 100, 300 or 1000 mg/kg/day, respectively, 5 days/week, for 2 years. Based upon mean body weight data, the total estimated lifetime dosages of TEA administered high dose group males and females were approximately 44 and 21 grams per animal, respectively, roughly two-thirds that administered orally to mice by Konishi *et al.*<sup>(7)</sup> Again, as in rats, an indeterminate amount of the test material would have been ingested during grooming. Vehicle control groups of mice were administered acetone only; however, no untreated controls were included in the study. Thus, as in the rat bioassay, the potential confounding effects of the acetone vehicle are unknown. The skin of mice appeared to tolerate the repeated exposure to concentrated solutions of TEA somewhat better than that of rats as chronic inflammation, but no necrosis, was observed in high dose group mice.

Despite the relatively high dosages of TEA employed in the mouse bioassay, no significant treatment-related changes in mortality or body weights, and relatively few toxic effects were observed. The most significant microscopic changes were found in the livers of male mice consistent with a chronic bacterial hepatitis (infection by *Helicobacter hepaticus*) in these animals. Chronic hepatitis in mice has been shown to cause liver tumors.<sup>(11)</sup> No microscopic evidence of bacterial infection was observed in liver tissues of most female mice following 15 and 24 months of dosing; however a research effort is presently underway to ascertain the potential infection of these animals using direct culture and PCR techniques (J. MacGregor in a personal communication).

The issue of potential tumorigenicity of chronically administered TEA in the mouse bioassays has primarily focused upon changes in the incidence of benign tumors (adenomas) of the liver and their potential relationship with bacterial hepatitis and or obesity in treated animals. The incidence of liver adenomas in male and female mice administered 2000 and 1000 mg/kg/day TEA, respectively, were observed to be numerically higher than in controls (54% vs. 74%, and 44% vs. 80%, respectively). Significantly, the incidence of

liver tumors in high dose group male mice was closely linked with evidence of bacterial infection. In fact, 94% of the mice in the study with evidence of infection also had tumors. In recognition of this "confounding factor," the authors concluded that these data did not present "clear evidence" of a carcinogenic response and considered the response in male mouse livers to be "equivocal."

A clear correlation between liver tumors and active bacterial hepatitis in high dose group female mice was not clearly identified during the study. However, given the potentially significant impact of an unrecognized or even resolved bacterial infection upon tumor formation in these animals, a retrospective evaluation of tissues from females for evidence of infection is currently underway (as noted). Another important factor in the interpretation of female mouse results is the potential impact of obesity. Liver tumor rates in mice display a strong association with body weight, especially at 12 months age.<sup>(11)</sup> Significantly, the 12-month old females in the study had higher body weights than comparable females in the historical data base (Avg. of 46 vs. 36 grams) and a higher incidence of liver adenomas, 44%, an incidence over double that observed in the only other dermal study with an acetone vehicle conducted at the testing facility (16%). Interestingly, a somewhat lower tumor incidence, roughly 33%, would have been expected in the present study based upon body weights alone. The potential impact of these known risk factors, upon tumor development coupled with the stress of repeated dermal dosing upon the results obtained is unclear. It was concluded that the liver tumor data in female mice, while not clear evidence of a tumorigenic response, still represented "some evidence" of carcinogenic activity of TEA.

An additional chronic dermal bioassay of TEA has been conducted in which the potential tumorigenicity of dermally administered "pure" TEA (99% purity) and "technical" TEA (80% purity) have been examined in mice by Kostrodymova *et al.*<sup>(13)</sup> Few details of this Soviet study are available, but it appears that TEA was applied to the backs of male CBA x C57Bl6 mice

as a 50% solution in acetone twice weekly for 14-18 months. The authors concluded that there was no direct tumorigenic activity of TEA.

## REFERENCES

1. **Benya, TJ; RD Harbison:** Aliphatic and Alicyclic Amines. In, *Patty's Industrial Hygiene and Toxicology*. Fourth Ed. Vol. 2. G. D. Clayton and F. E. Clayton, Eds. J. Wiley & Sons, Inc., NY, 1994. pp. 1087-1175,
2. **BIBRA:** *Toxicology Profile: Triethanol-amine*. BIBRA Toxicology International, Surry, GB. (1990).
3. **BIBRA:** *Toxicology Profile: Ethanolamine*. BIBRA Toxicology International, Surry, GB. (1993).
4. **BIBRA:** *Toxicology Profile: Diethanolamine*. BIBRA Toxicology International, Surry, GB. (1993).
5. **CTFA:** Final Report of the Safety Assessment for Triethanolamine, Diethanolamine and Monoethanolamine. *J. Am. Col. Toxicol.* 2: 183-235 (1983)..
6. **Maekawa, A; H Onodera, H Tanigawa, K Furuta, J Kanno, C Matsuoka, T Ogiu, and Y Hayashi:** Lack of carcinogenicity of triethanolamine in F344 rats. *J. Toxicol. Environ. Hlth.* 19: 345-357 (1986).
7. **Konishi, Y; A Denda, K Uchida, Y Emi, H Ura, Y Yokose, K Shiraiwa, and M Tsutsumi:** Chronic toxicity and carcinogenicity studies of triethanolamine in B6C3F1 mice. *Fund. Appl. Toxicol.* 18: 25-29 (1992).
8. **Hoshino, H; H Tanooka:** Carcinogenicity of triethanolamine in mice and its mutagenicity after reaction with sodium nitrite in bacteria. *Cancer Res.* 38: 3918-3921 (1978).
9. **Hejtmancik, M; JD Toft, RL Persing, and RL Melnick:** Two-year dermal study of triethanolamine in F344 rats and B6C3F1 mice. *The Toxicol.* 15: 202 (Abstr.) (1995).

10. **National Toxicology Program:** Toxicology and Carcinogenesis Studies of Triethanolamine in F344/N Rats and B6C3F1 Mice. TRP TR #449, NIH Pub. No. 94-3365, Draft Report (1994).
11. **Ward, JM; JG Fox, MR Anver, DC Haines, CV George, MJ Collins, PL Gorelick, K Nagashima, MA Gonda, RV Gilden, JG Tully, RJ Russell, RE Benveniste, BJ Paster, FE Dewhirst, JC Donovan, LM Anderson, and JM Rice:** Chronic active hepatitis and associated liver tumors in mice caused by a persistent bacterial infection with a novel Helicobacter species. *J. Natl. Cancer Inst.* 86: 1222-1227 (1994).
12. **Seilkop, SK:** The effect of body weight on tumor incidence and carcinogenicity testing in B6C3F1 mice and F344 rats. *Fund. Appl. Toxicol.* 24: 247-259 (1995).
13. **Kostrodymova, GM; VM Voronin, and NN Kostrodymova:** The toxicity and the possibility of carcinogenic and co-carcinogenic properties of triethanolamines [English Translation]. *Gig. Sanit.* 3: 20-25 (1976).

## **Biocide Toxicity: A Comparison of the Toxicological Properties of Common Metalworking Fluid Biocides**

**Frederick J. Passman**

Biodeterioration Control Associates, Inc. P.O. Box 268176, Chicago, Illinois 60626-8176

### **ABSTRACT**

After creating a contextual understanding of the rationale for biocide use and toxicological relationship between biocides and non-biocide industrial chemicals, the author compares the relative toxicity of eleven US EPA registered biocides. Products included in this survey represent four product classes: formaldehyde; formaldehyde-releasing, formaldehyde-condensates; non formaldehyde-releasing, formaldehyde-condensates, and non-formaldehyde products.

Acute and sub-chronic toxicity, skin sensitization, eye irritation, carcinogenicity, mutagenicity, teratogenicity and wildlife data comparisons demonstrate that biocides cannot be differentiated by their toxicological profiles. Toxicological profiles are product specific. Active ingredients that are relatively non-toxic by one criterion, may be highly toxic by other criteria. Clustering biocides, based on the involvement of formaldehyde in their manufacture or function is inappropriate. Instead, product-specific toxicological, volatility and solubility data must be coupled to performance data and application design parameters. This approach ensures that biocides are used to provide maximum performance benefits with minimum employee health and safety risk.

### **INTRODUCTION**

#### **Why Use Biocides?**

Fifty years have passed since early researchers first established the relationship between uncontrolled microbial contamination and metalworking fluid rancidity.<sup>(1)</sup> Biocides are used in metalworking fluids to control microbial contamination. Uncontrolled microbial contamination affects fluid performance and the shop atmosphere. Fluid biodeterioration symptoms include slime

production, emulsion destabilization and noxious, malodorous gas evolution; evoking images of back-up cesspools and swamps. Endotoxins, a component of the cell "wall" of most of the bacteria that proliferate in metalworking fluids are implicated in sick-building syndrome.<sup>(2)</sup> As recently as the mid-1980's, typical coolant-life, in recirculating systems, was 12 - 16 weeks (one plant manager opined that before he had started monitoring for microbial contamination, he had never had microbial problems, he just dumped his systems whenever he heard too many odor complaints).

Biocides are chemicals used specifically to control microbial contamination in products. The Federal Insecticide and Rodenticide Act (FIFRA) provides comprehensive guidance on the manufacture, registration and use of industrial biocides, including those used to preserve and disinfect metalworking fluids. Since FIFRA regulations require considerable precautionary, use and disposal information be included on biocide labels, these products are often perceived as presenting unique health and safety risks to metalworking industry employees. In fact, the toxicological profiles of most industrial biocides are not substantially different from that of other industrial chemicals used routinely in manufacturing plants.

During the 1970's, Congress passed the Clean Water Act and Resource Conservation and Recovery Act. However, it was not until the late 1980's as compliance deadlines drew near that the race to minimize industrial waste became serious. The economics of waste treatment and disposal differ dramatically from a decade ago. In most States, spent metalworking fluids are classified as hazardous wastes. Haulers charge as much as \$3.00/gallon to remove spent fluids from machine

shops. To control waste costs plant managers, working with their coolant suppliers and system operators, have learned how to extend coolant life dramatically. Improved programs for rancidity control have extended system shut-down intervals (due to coolant problems) to multiple-years. Better waste-solids removal systems and contamination monitoring programs contributed substantially to improved coolant life. But strategic biocide application was the single most important element that drove the revolution.

**Biocides versus Bioresistant Additives:** Today, biocides are under increased scrutiny because they are clearly identified as toxic chemicals. Regulated under FIFRA, biocide product labels contain much more information than labels of other, equally hazardous industrial chemicals. Unfortunately, more information is often interpreted as reflecting greater risk. Capitalizing on the climate of fear, some vendors are offering *biostatic* or *bioresistant* additives, for which the only performance data available are biocidal activity. Since the toxicological data have not been developed, and their products have not been approved as biocides under FIFRA, manufacturers can claim that their bioresistant additives are less toxic than registered chemistries. Any chemical whose primary function is to inhibit microbial activity and/or proliferation is a biocide. If it is not registered under FIFRA, it is an illegal biocide.

**Risk Analysis:** A triad of factors dictates metalworking fluid biocide selection and use. Technological considerations determine whether microbial contamination control is needed, and which options are likely to be most effective. Fiscal considerations provide the basis for evaluating the relative cost-effectiveness of contamination control alternatives. Risk determination considers the human factors (toxicology and exposure) associated with alternative contamination control strategies. Carefully assessed risk, when balanced against cost, supports risk-benefit analysis. In an ideal world, antimicrobials that present the most favorable risk/cost-benefit profile are the ones selected for

use. Unfortunately, in the absence of objective data, emotional considerations often drive biocide selection and application decisions.

The metalworking fluid environment is a particularly challenging one in which to attempt to assess the risk attributed by any individual chemical. Most metalworking fluid base stocks are poorly characterized distillation fractions. Little research has addressed the toxicology of metalworking fluid blends and the changes to that toxicology as coolant ages in a recirculating metalworking fluid system. This Symposium is a tribute to our industry's sincere desire to assess our present level of knowledge.

Since industrial biocides are officially classified as toxic chemicals, they receive special scrutiny by system operators and managers responsible for coolant performance and additive use. Increased concerns regarding workplace formaldehyde (HCHO) exposure have drawn considerable attention to one particular group of anti-microbials; the HCHO-condensate biocides. Recent OSHA hazard communications requirements for HCHO exposure reporting have exacerbated industry concerns, despite the fact that only aldehyde-chemistry biocides denature endotoxins,<sup>(3)</sup> the cell component implicated in sick-building syndrome.

**Formaldehyde-condensate biocides:** As their name implies, formaldehyde-condensate biocides include HCHO as one of the reactants used in their manufacture. Typically, HCHO reacts with an amine (for example, mono-ethanolamine; MEA) to form a new chemical compound

$$3 \text{ HCHO} + 1 \text{ MEA} \longrightarrow 1 \text{ hexahydro-1,3,5 tris (hydroxyethyl)-s-triazine; HETRIAZ}.$$

Since HCHO was identified as a suspect carcinogen, in the early 1980's, HCHO-condensate biocides have received increased attention. As an industry, we assume that HCHO exposure, in our machine shops, originates from HCHO-condensate biocides.

Dr. Cohen will speak to the issue of HCHO-exposure during his presentation, tomorrow. My paper focuses on the relative toxicological properties of four groups of anti-microbials. The

first group includes HCHO, alone. Five HCHO-condensate biocides comprise the second group. The biocidal activity of these four products depends on formaldehyde release.<sup>(4)</sup> The third group includes two HCHO-C biocides that do not

rely on HCHO-release to function. Three non-HCHO biocides form the final group. Table 1 lists the biocides included in this paper.

**Table 1. Biocides compared in this paper**

Abbreviation	Primary Active Ingredient(s)	Formaldehyde Condensate
BNPD	2-bromo-2nitro-1,3,-propanediol	Yes
CMIT	5-chloro-2-methyl-4-isothiazolin-3-one + 2-methyl-4-isothiazolin-3-one	No
DOTO	4,4-dimethyloxazolidine + dioxabicyclo(3.3.0) octane + 5-hydroxymethyl-1-aza-3,7- dioxabicyclo(3.3.0) octane + 5-hydroxypoly (methyleneoxy-methyl-1-aza-3,7-dioxabicyclo(3.3.0) octane	Yes
GLUT	Glutaraldehyde	No
HCHO	Formaldehyde	N/A
HDO	5-hydroxymethoxymethyl-1-aza-3,7-3,4,4-trimethyloxazolidine	Yes
HETRIAZ	Hexahydro-1,3,5-tris (2-hydroxyethyl)-s-triazine	Yes
NMEND	4-(2-nitrobutyl)morpholine + 4,4'-(2-ethyl-2-nitrotrimethylene) dimorpholine	Yes
OPP	O-phenylphenol	No
PED	Poly[oxyethylene(dimethyl imino)ethylene dichloride	No
TETRIAZ	Hexahydro-1,3,5-triethyl-s-triazine	Yes
TN	2-hydroxymethyl-2-nitro-1,3-propanediol	Yes

**Data Analysis:** All data were provided by product manufacturers. Parameters for which quantitative data were available were analyzed statistically, using one-way analysis of variance (ANOVA). Sub-chronic and avian wildlife

toxicity data were compared subjectively.

## DATA AND DISCUSSION

### Acute Toxicity

Acute oral toxicity (LD<sub>50</sub>), dermal toxicity (LD<sub>50</sub>), acute inhalation toxicity (LC<sub>50</sub>), eye

irritation, skin sensitization and skin irritation data were compared among all biocides. For all parameters, variation among products within a group was greater than variation among biocide groups. The exception was that formaldehyde was substantially more toxic (acute oral LD<sub>50</sub> (HCHO)= 100 mg/kg body weight; acute dermal LD<sub>50</sub> (HCHO)= 420 mg/kg body weight) than any other product evaluated. As expected, inhalation toxicity correlated well with product volatility. Low vapor pressure products had lower acute inhalation LC<sub>50</sub>'s than did higher vapor pressure products. The most toxic product was HCHO (LC<sub>50</sub> = 0.2 mg/liter; 10-hour exposure).

**Human Skin Sensitization:** Only HCHO and GLUT are rated as sensitizers in both animal and human tests, however, at least one product in each of the four groups is a sensitizer in either animal or human skin patch tests. Consequently, the biocide groups cannot be differentiated on the basis of product sensitization potential.

**Sub-chronic Toxicity:** As with acute toxicity parameters, sub-chronic dermal toxicity varied more within biocide groups than among them. For HCHO-release biocides, no observable effect levels (NOEL's) ranged from 5 (DOTO) to >1,000 (TN) mg/kg/day. The NOEL range for non-formaldehyde products was 0.4 (CMIT) to 25,000 (GLUT) mg/kg/day.

**Genotoxicity:** Genotoxicity data are summarized in Table 2. The inter-group data pattern was consistent with that noted for other toxicity data. HCHO was Ames-test positive for mutagenicity; as were CMIT (a non-HCHO biocide), and TETRIAZ (HCHO-condensate; HCHO-release). No generalizations could be made about biocide group mutagenicity or teratology. However the highest non-HCHO biocide teratological NOEL was lower, by a factor of three, than the lowest HCHO-release biocide NOEL.

**Table 2. Genotoxicity of 11 Selected Biocides**

Biocide	Mutagenicity/Genotoxicity				Teratology		
	Ames	<i>In vitro</i> Mouse Lymphoma	Chinese Hamster Ovary	Rat Liver	Unscheduled DNA Synthesis	Terato- genicity	NOEL (mg/kg/ day)
BNPD	Neg	N.A. <sup>1</sup>	Neg	Neg	N.A.	Neg	>40
CMIT	Pos <sup>2</sup>	Pos	Neg	N.A.	Neg	Neg	< 2.0
DOTO	Neg	Equivocal <sup>3</sup>	Wk. Pos.	N.A.	N.A.	N.A.	N.A.
GLUT	Neg	N.A.	Neg	Neg	Neg	Neg	25
HCHO	Pos	Pos	Pos	Pos	Pos	Neg	74
HDO	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
HETRIAZ	Neg	N.A.	Neg	N.A.	Neg	Neg	250
NMEND	Neg	N.A.	Neg	N.A.	Neg	N.A.	N.A.
PED	Neg	Neg	Neg	Neg	Neg	Neg	>125
TETRIAZ	Pos	Equivocal	Equivocal	Equivocal	Pos	Neg	75
TN	Neg	N.A.	Neg	Neg	Neg	Neg	375

**Notes:**

<sup>1</sup>N.A. : Data not available.

<sup>2</sup>Positive for *Salmonella typhimurium* strain TA 98, without rat S9 activator.

<sup>3</sup>Equivocal: inconsistent test results among repeated studies.

**Aquatic Wildlife Toxicity:** The only test species for which there were sufficient data, for inter-group comparisons, were bluegill sunfish and rainbow trout. Again, intra-group variation was significantly greater than inter-group variation. However, the average HCHO-release biocide aquatic wildlife toxicity was substantially lower than that of the other two groups

( $LC_{50} \text{ (HCHO-release)} = 1,300 \pm 2,900$ ;  $LC_{50} \text{ (HCHO- non-release)} = 20 \pm 22$ ;  $LC_{50} \text{ (non-HCHO)} = 12 \pm 15 \text{ mg/liter}$ ; no data for HCHO).

**Avian Wildlife Toxicity:** Inter-group comparisons for eight-day dietary  $LC_{50}$  data for bobwhite quail and mallards were complicated by the failure of most studies to report actual end points. It's meaningless to compare an  $LC_{50}$  of  $> 60$  ppm (CMIT) with one reported as 779 ppm (BNPD) or  $> 10,000$  ppm (GLUT). Bobwhite quail  $LC_{50}$  data for non-HCHO biocides ranged from  $> 60$  ppm to  $> 10,000$  ppm. All HCHO-release product manufacturers reported  $LC_{50} \text{ (bobwhite quail)} > 5,000$  ppm for their products. The  $LC_{50}$ 's for BNPD and NMEND, respectively were 779 and 5,620 ppm. Mallard data followed a similar pattern.

## DISCUSSION AND CONCLUSIONS

Industrial biocides are toxic by design. Unlike other, equally toxic industrial chemicals (strong acids, caustic, solvents, etc.) their primary value depends on their ability to kill microbes without adversely affecting other coolant performance properties. The benefits derived from biocide use must be balanced against the economic and health impacts.

Although there is general consensus regarding the link between microbial contamination and product rancidity<sup>(5)</sup> the same is not true regarding the role of metalworking fluid microbes and employee health.<sup>(6)</sup> However, a growing body of data suggest that uncontrolled microbial growth may, in fact, present a significant health risk.<sup>(7,8)</sup> Considering then the economic and potential health risks associated with uncontrolled microbial contamination, one can make a strong argument

that the benefits, associated with biocide use, outweigh the risks. The caveat here is using appropriate products in appropriate applications.

Toxicological dose responses typically are not linear. That is, if a chemical's  $LD_{50} = 1,000$  mg/kg, it does not necessarily follow that 250 mg/kg will kill 25-percent of the test population. This concept is important to understand. Many industrial biocide active ingredients are also used routinely as preservatives in personal care products, food packaging materials and other "intimate contact" applications. In these products the potential for biocide ingestion and absorption is much greater than it is through incidental contact with metalworking fluids. Consequently, the risk lies in misuse rather than incidental exposure due to biocide use in accordance with manufacturer's instructions.

The toxicological data provide one basis for matching products with intended applications; and should be considered in context with solubility, volatility, half-life, efficacy and treatment cost data. Skin sensitizing products may be used risk-free, if they are built into metalworking fluid concentrates, eliminating the possibility of direct contact at the plant. Volatile products are best suited for use in low-mist, low heat applications. Broad category monikers, such as *HCHO-condensate biocide* are virtually meaningless. Manufacturing personnel responsible for coolant formulation or coolant system maintenance should be wary of claims that either promote or condemn biocide groups, based on toxicological properties. As with all industrial products the risk associated with biocides lies mostly in product abuse, not use.

## REFERENCES

1. **Lee, M; AC Chandler:** A Study of the Nature Growth and Control of Bacteria in Cutting Compounds. *J. Bacteriol.* 41: 373-386 (1941).
2. **Burrell, R; Y Shu-Hua:** Toxic Risks from Inhalation of Bacterial Endotoxin. *Brit. J. Indust. Med.* 47: 688-691 (1990).
3. **Douglas, H; HW Rossmore, FJ Passman, and LA Rossmore:** Evaluation of Endotoxin-

- Biocides Interaction by the *Limulus* Amoebocyte Assay. *Devel. Indust. Microbiol.* 31: 221-224 (1990).
4. **Sondossi, M; HW Rossmore, and JW Wireman:** The Effect of Fifteen Biocides on Formaldehyde-Resistant Strains of *Pseudomonas aeruginosa*. *J. Indust. Microbiol.* 1: 87-96 (1986).
  5. **Holtzman, GH; HW Rossmore, E Holodink, and M Weintraub:** Interrelationships Between Biodeterioration, Chemical Breakdown, and Function in Soluble Oil Emulsions. *Devel. Indust. Microbiol.* 23: 207-216 (1982).
  6. **Rossmore, HW:** Biostatic Fluids, Friendly Bacteria and Other MYTHS in Metalworking Microbiology. *Lub. Eng.* 50: 253-260 (1993).
  7. **Elsmore, R:** The Survival of *Legionella pneumophila* in Dilute Metalworking Fluids. *Tribol. Internat.* 22: 213-217 (1989).
  8. **Kennedy, SM:** Acute Pulmonary Responses Among Automobile Workers Exposed to Aerosols of Machining Fluids. *Amer. J. Indust. Med.* 15: 627-641 (1989).

## Use of a Bioassay to Evaluate the Respiratory Irritancy of Metalworking Fluids and Their Components

**Michelle M. Schaper** and Katherine A. Detwiler-Okabayashi  
University of Pittsburgh, Graduate School of Public Health  
Center for Environmental and Occupational Health and Toxicology  
260 Kappa Drive, Pittsburgh, PA 15238

### ABSTRACT

Mice were exposed to aerosols of seven metalworking fluids (MWFs) and the components used to formulate these fluids. During single 180-minute exposures, the sensory and pulmonary irritating properties of each fluid and component were evaluated. Typically, the MWFs and components evoked sensory and pulmonary irritation in mice, with sensory irritation occurring earlier in the exposures and pulmonary irritation occurring later in the exposures. At the time of maximum response, the predominant respiratory effect of the aerosols was pulmonary irritation. As a result of this irritation, respiratory frequency ( $f$ ) decreased. Using the measurements of  $f$ , concentration-response relationships were developed for the aerosols. From such relationships, the concentration capable of producing a 50% decrease in  $f$  ( $RD_{50P}$ ) was obtained for each MWF and component. By dividing the  $RD_{50P}$  values by 60, occupational exposure limits (OELs) were suggested. These limits, which were generally in the range of 2-10 mg/m<sup>3</sup>, would prevent pulmonary irritation in workers. The bioassay used in this study will be valuable in evaluating the respiratory irritancy of other MWFs and their components, and readily identifies individual components that are potent sensory and/or pulmonary irritants. For MWFs, it also facilitates assessment of the relative potency of these mixtures as irritants.

### INTRODUCTION

It has been estimated that over 10 million workers in the United States are exposed to metalworking fluids (MWFs).<sup>(1)</sup> Such workers are involved in numerous industries and they perform

a wide range of operations including cutting, drilling, grinding, and milling. In these operations, MWFs serve as lubricants and coolants. In the past, MWFs were usually petroleum-based oils (i.e., straight oils) and were often described as "cutting oils" or "cutting fluids." Today there are more types of MWFs available for use in the workplace. The traditional straight oils may still be found, but there are also soluble fluids (30-85% v/v oil), semi-synthetic fluids (5-30% v/v oil), and synthetic fluids (no oil).<sup>(2)</sup>

The two principal routes of occupational exposure to MWFs are dermal (skin) and inhalation (respiratory tract). When MWFs are used in operations requiring pressure and/or high temperature, aerosols whose size (i.e., mass median aerodynamic diameter) is below 10  $\mu$ m may be formed. In some instances, the particle (droplet) size may be even less than 1  $\mu$ m.<sup>(3,4)</sup> These aerosols may be inhaled by workers and may stimulate nerve endings in the respiratory tract, thereby evoking a variety of respiratory reactions. It is also possible for deposited aerosols to pass into the circulatory system and gain access to other target organs. There has been increasing concern regarding the potential human health effects that may occur as a result of inhalation exposure to aerosols of MWFs. Such exposures may occur on an acute basis, but there are many examples of chronic exposure to MWFs in the American workforce.

Previous toxicological studies have focused on straight oils and generally the investigators have evaluated changes in pulmonary histopathology following inhalation exposure. Interestingly, few studies have been published in

this area between 1950 and 1990. Thus, there is little known about the potential respiratory effects of newer types of MWFs (containing little or no oil) or the components used to formulate these fluids. Furthermore, there is little guidance regarding occupational exposure limits (OELs) for newer types of MWFs or their components.<sup>(5,6)</sup> For these reasons, the present study was undertaken.

## METHODS

### Metalworking Fluids and Their Components

Seven "neat" MWFs (i.e., not yet used or

contaminated) were included in this study. As summarized in Table 1, there were 4 soluble fluids (C, D, E, G), 1 semi-synthetic fluid (B), 1 synthetic fluid (A) and 1 straight oil (F). Additionally, the components from three of these MWFs (A, B, E) were studied here and are listed in Table 2. The MWFs and components were obtained from the United Auto Workers (UAW) and General Motors Corporation (GM) National Joint Committee on Health and Safety. The fractional composition of the MWFs has been given previously.<sup>(7 through 15)</sup>

Table 1. Comparison of RD<sub>50</sub> Values for the Seven MWFs<sup>1</sup> Studied Vs. Those for Known Irritants

Chemical or Mixture	RD <sub>50</sub> P (or RD <sub>50</sub> TC) <sup>3</sup> (mg/m <sup>3</sup> )	Occupational Exposure Limit <sup>4</sup> (mg/m <sup>3</sup> )
MWF A (synthetic fluid)	119	2.00
MWF B (semi-synthetic fluid)	154	2.60
MWF G (soluble fluid)	452	7.50
MWF D (soluble fluid)	472	7.90
MWF E (soluble fluid)	497	8.30
MWF C (soluble fluid)	683	11.40
MWF F (straight oil)	325,000	>1,000
Chlorine	36 (12.4 ppm)	0.60 (0.2 ppm)
Acrolein	326 (142 ppm)	5.40 (2.4 ppm)
Nitrogen Dioxide	647 (344 ppm)	0.80 (5.7 ppm)
Hydrogen Chloride	806 (540 ppm)	3.40 (9.0 ppm)
Sulfur Dioxide	996 (380 ppm)	16.60 (6.3 ppm)
Ammonia	1115 (1603 ppm)	18.60 (26.7 ppm)

<sup>1</sup>From Reference 7.

<sup>2</sup>From Reference 26.

<sup>3</sup>RD<sub>50</sub>TC refers to the concentration capable of producing a 50% decrease in *f* in tracheally-cannulated mice.

<sup>4</sup>Each RD<sub>50</sub> value was divided by 60 to suggest an occupational exposure limit that would prevent *pulmonary irritation* in workers.

Table 2. RD<sub>50</sub> Values for Components in MWFs A, B, and E<sup>1</sup>

MWF	Component	RD <sub>50</sub> P (mg/m <sup>3</sup> )	Occupational Exposure Limit <sup>2</sup> (mg/m <sup>3</sup> )
E	Sodium Sulfonate	102	1.7
B	Sodium Sulfonate	103	1.7
E	Tall Oil Fatty Acids	105 <sup>3</sup>	3.2 <sup>3</sup>
B	Potassium Soap	129	2.2
B	S-Triazine, 1,3,5[2H,4H,6H]-Triethanol	137	2.3
B	Tall Oil Acid Diethanolamide	155	2.6
A	Fatty Acid Alkanolamide Condensates	190	3.2
A	Hexahydro-1,3,5 Tris(2-Hydroxyethyl)-S-Triazine	190	3.2
B	Caprylic Acid Diethanolamide	197	3.3
A	Tolutriazole	205	3.4
A	Phosphonate Sequestrant	330	5.5
A	Isononanoic Acid	420	7.0
A	Monoisopropanolamine	440	7.3
A	2-Amino-2-Methyl-1-Propanol	640	10.7
A	Sodium Pyrithione	740	12.3
A	Triisopropanolamine	815	13.6
B	Petroleum Oil	3,188	53.1
A	Diisopropanolamine	3,200	53.3
E	Paraffinic Oil	5,437	90.6

<sup>1</sup>From References 9-15.

<sup>2</sup>Each RD<sub>50</sub>P value was divided by 60 to suggest an occupational exposure limit that would prevent *pulmonary irritation* in workers.

<sup>3</sup>The RD<sub>50</sub>S value for the tall oil fatty acids was divided by 33 to suggest an occupational exposure limit that would prevent *sensory irritation* in workers.

### **Mouse Bioassay**

In this study, a mouse bioassay was used for recognition of the sensory (i.e., eye, nose, throat) and pulmonary (i.e., deep lung) irritating properties of inhaled MWFs and their components.<sup>(16-22)</sup>

Briefly, mice exposed to sensory irritants exhibit a reflex inhibition of respiration with a lengthening of expiration. This delay during expiration has also been termed as "braking". As the time of braking (TB) increases, respiratory frequency (*f*) decreases. In contrast, mice exposed to pulmonary irritants exhibit pauses between breaths. As the length of the pause (TP) increases, respiratory frequency (*f*) decreases. With both sensory and pulmonary irritants, these effects on TB, TP, and *f* are dependent upon exposure concentration. That is, the higher the exposure concentration, the

greater the increase in TB or TP and thus, the greater the decrease in *f*. In this manner, it is possible to develop concentration-response relationships for chemicals possessing sensory and/or pulmonary irritating properties.

Based upon such concentration-response relationships, it is possible to determine the concentration that is capable of producing a 50% decrease in mean *f*. This has been termed the RD<sub>50</sub>.<sup>(16,18,23)</sup> In the original definition, this decrease in *f* was due to *sensory irritation*. Recently a descriptor, "S" or "P", has been added after RD<sub>50</sub> to designate whether the decrease in *f* (at the time of maximum response) is predominantly due to sensory or pulmonary irritation.<sup>(13-15)</sup> Thus, RD<sub>50</sub>S or RD<sub>50</sub>P values are now reported when using the bioassay to evaluate

the irritating properties of MWFs or their components.

### **Human Extrapolation From Mouse Bioassay**

It has been shown that humans exposed to a sensory irritant, at its  $RD_{50S}$ , would experience intolerable burning of the eyes, nose, and throat.<sup>(18,23)</sup> To prevent these effects in occupational settings, an acceptable exposure level may be predicted by dividing the  $RD_{50S}$  by 33 (or  $0.03 \times RD_{50S}$ ). Indeed, an excellent correlation has been demonstrated for 89 industrial chemicals between their Threshold Limit Values<sup>(5)</sup> (TLVs) and  $0.03 \times RD_{50S}$  values.<sup>(18,23)</sup> For pulmonary irritants, such a relationship between the TLVs and  $RD_{50P}$  values has not been established. It has been suggested that the  $RD_{50P}$  be divided by 60 to arrive at a concentration that would prevent pulmonary irritation in workers.<sup>(24,25)</sup> Because pulmonary irritants may produce hemorrhage and edema in the lung, and even death,<sup>(16)</sup> it is reasonable to introduce a larger "safety factor" (60 vs. 33) when proposing OELs. These limits will serve as good starting points for controlling worker exposures.

### **Generation and Characterization of Inhalation Exposure**

Each MWF or component was infused via a syringe pump into a Pitt No.1 or No.4 aerosol generator<sup>(7-15,17)</sup> whose output was directed into the mouse exposure chamber. Sham exposures were conducted similarly with distilled water. During aerosolization of distilled water, MWFs, or components, air samples were collected from the mouse exposure chamber onto glass fiber filters. The exposure concentration was then determined via gravimetric analysis. There were no changes in the weight of filters used for sampling during exposure to distilled water. This indicated that water mist initially produced from the aerosol generator had vaporized. No further assessment of water vapor concentration was conducted. Appreciable vaporization also occurred with 2-amino-2-methyl-1-propanol which was a component in MWF A, and exposure concentrations were determined using infrared spectroscopy.<sup>(11,15)</sup> Thus, with the exception of 2-

amino-2-methyl-1-propanol, only solid particulates and/or particulates having a low vapor pressure were captured on filters. Water was excluded in the gravimetric analyses.<sup>(7-15)</sup>

A Marple personal cascade impactor was used for sizing the aerosols.<sup>(7-15)</sup> For the majority of MWFs and components evaluated in this study, the mass median aerodynamic diameter (MMAD) was 1-2  $\mu\text{m}$  and the geometric standard deviation ( $\sigma_g$ ) was approximately 2.<sup>(7-15)</sup>

### **Experimental Protocol**

The experiments were 220 minutes in length, consisting of a 20-minute pre-exposure (air only), a 180-minute exposure (sham, MWF, or component), and a 20-minute recovery post-exposure (air only). Two types of sham exposures were conducted using air only or water vapor. The remaining exposures were conducted using MWFs or components of MWFs.

## **RESULTS**

### **Respiratory Effects of Sham-Exposure**

No changes in breathing patterns or  $f$  occurred in mice during 180-minute exposure to air only or to water vapor.

### **Respiratory Effects of MWF or Component Exposure**

All of the MWFs evaluated in this study evoked both sensory and pulmonary irritation in mice, with sensory irritation occurring earlier in the 180-minute exposures and pulmonary irritation occurring later in these exposures. At the time of maximum response, the predominant respiratory effect of the MWFs was pulmonary irritation. As a result of this irritation, respiratory frequency ( $f$ ) decreased. Using the measurements of mean  $f$ , a concentration-response relationship was developed for each MWF. From such relationships, the concentration capable of producing a 50% decrease in mean  $f$  ( $RD_{50P}$ ) was obtained for each MWF. These values, ordered from smallest to largest, are listed in Table 1. In terms of relative potency as respiratory irritants, synthetic and semi-synthetic fluids (A,B) were more potent than the soluble fluids (C,D,E,G). Both of these types of fluids

were much more potent than the straight oil (F).

Like the MWFs themselves, the components in MWFs A, B, and E generally evoked sensory and pulmonary irritation. The major exceptions to this finding were the sulfonates in MWFs A and B and the tall oil fatty acids in MWF E. The tall oil fatty acids produced predominantly sensory irritation whereas the two sulfonates produced little sensory irritation and a rapid onset of pulmonary irritation. With each component, decreases in  $f$  occurred in a concentration-dependent manner and it was possible to develop a concentration-response relationship for the components in MWFs A, B, and E. Their  $RD_{50}$  values are given in Table 2. With the exception of the tall oil fatty acids, they are  $RD_{50}P$  values since pulmonary irritation was observed at the time of maximum response during the 180-minute exposures. The values in Table 2 are also ordered from smallest to largest. Among the most potent components were the sulfonate, soaps, triazine, and alkanolamides, which indicated their important contribution in defining the observed irritancy of MWFs A, B, and E. The isopropanolamines were moderately potent while the straight oils were among the least potent components.

Of all the MWFs and components evaluated in this study, only the triazine in MWFs A and B (see Table 2) produced delayed animal deaths. These deaths generally occurred 24-72 hours following a single exposure to triazine at concentrations above approximately  $150 \text{ mg/m}^3$ .

### **Proposal of Occupational Exposure Limits for MWFs and Their Components**

Each of the  $RD_{50}P$  values for the seven MWFs was divided by 60 to suggest an OEL to prevent pulmonary irritation. As noted in Table 1, the proposed limits range from approximately  $2\text{-}10 \text{ mg/m}^3$ . Also listed in Table 1 are 6 industrial chemicals whose *sensory* irritating properties have been well-established.<sup>(18,23,26)</sup> To evaluate their *pulmonary* irritating properties alone, mice were tracheally-cannulated (TC) and exposed to the same chemicals, thus eliminating possible oronasal absorption and providing direct delivery to the deep lung.<sup>(26)</sup> Concentration-response

relationships were developed for these chemicals and  $RD_{50}TC$  values were obtained. Conceptually, the  $RD_{50}TC$  and  $RD_{50}P$  values are similar to one another; both imply that the decrease in  $f$  is due to pulmonary irritation. The  $RD_{50}TC$  concentrations, originally expressed in ppm, have been converted to  $\text{mg/m}^3$  for comparison purposes. Thus, it is easy to see that the potency of the MWF aerosols, as pulmonary irritants, was comparable to that of acrolein (vapor) or nitrogen dioxide (gas). As done with  $RD_{50}P$  values for the MWFs,  $RD_{50}TC$  values were divided by 60 to suggest OELs. Thus, the OELs for acrolein and nitrogen dioxide are similar to those for the MWFs.

OELs were also proposed for each component evaluated in this study. With the exception of the tall oil fatty acids in MWF E, the remaining components evoked pulmonary irritation at the time of maximum response and thus their  $RD_{50}P$  values were divided by 60 to arrive at the values given in Table 2. Because the tall oil fatty acids evoked mainly sensory irritation at the time of maximum response, its  $RD_{50}S$  was divided by 33 to obtain an OEL. Like the MWFs, the predicted OELs for the components were generally in the range of  $2\text{-}10 \text{ mg/m}^3$  which are similar to OELs for acrolein and nitrogen dioxide.

### **CONCLUSIONS**

This study has demonstrated that the mouse bioassay may be used to evaluate the sensory and pulmonary irritating effects of complex mixtures such as MWFs and their individual components. For the 7 MWFs and 19 components, the predominant respiratory effect was pulmonary irritation. Based on decreases in  $f$  that occurred during exposures, concentration-response relationships were developed and  $RD_{50}P$  (or  $S$ ) values were obtained. Using these  $RD_{50}$  values, the relative potency of the MWFs and components, as irritants, was estimated. Thus, the fluids and components which are the most (and least) irritating have been identified. With this information, it may then be possible to discontinue use of particular fluids, to re-formulate existing fluids, or to develop new fluids.

For each MWF and component evaluated in

the present study, OELs were proposed to protect workers from their pulmonary irritating effects. At this time, there is little guidance from the Occupational Safety and Health Administration (OSHA), the Environmental Protection Agency (EPA), the American Conference of Governmental Industrial Hygienists (ACGIH) or any other agency or group regarding acceptable workplace concentrations to these MWFs or components. Clearly, the aerosolized MWFs are not oil mists and the 5 mg/m<sup>3</sup> Permissible Exposure Limit<sup>(6)</sup> (PEL) or Threshold Limit Value<sup>(5)</sup> (TLV) is inappropriate. Thus, our proposed limits of 2-10 mg/m<sup>3</sup> will serve as good starting points for controlling worker exposure to the MWFs and components included in this study.

The mouse bioassay will be useful in evaluating the sensory and pulmonary irritating effects of other MWFs and components, thereby increasing the existing database. As noted above, it will permit recognition of biologically "active" components which should assist the manufacturers who formulate MWFs. The toxicological data will also be valuable to those individuals involved with selection of MWFs for use in the workplace. Ultimately, these data will be used by a variety of professionals who must provide hazard communication to workers and who are responsible for establishing guidelines and strategies to protect the health and safety of workers.

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## REFERENCES

1. **Jackson, K:** Machining fluids linked to cancer. *Automotive News*: July 27, 1992.
2. **Mackerer, CR:** Health effects of oil mists, a brief

- review. *Toxicol. Ind. Health* 5: 429-440 (1989).
3. **Chan, TL; JB D'Arcy, and J Siak:** Size characteristics of machining fluid aerosols in an industrial environment. *Appl. Occup. Environ. Hyg.* 5: 162-170 (1990).
4. **Kennedy, SM; IA Greaves, D Kriebel, EA Eisen, TJ Smith, and SR Wolskie:** Acute pulmonary responses among automobile workers exposed to aerosols of machining fluids. *Am. J. Ind. Med.* 15: 627-641 (1989).
5. **American Conference of Governmental Industrial Hygienists:** 1994-1995 Threshold Limit Values (TLVs) for Chemical Substances and Physical Agents and Biological Exposure Indices (BEIs). Cincinnati, OH: American Conference of Governmental Industrial Hygienists, 1994.
6. **U.S. Dept. of Labor. Occupational Safety and Health Administration:** *Air Contaminants -- Permissible Exposure Limits.* Washington, D.C.: Title 29 Code of Federal Regulations, Part 1910.1000 (1989).
7. **Schaper, MM; K Detwiler:** Evaluation of the acute respiratory effects of aerosolized machining fluids in mice. *Fund. Appl. Toxicol.* 16: 309-319 (1991).
8. **Detwiler, K; MM Schaper:** Further evaluation of pulmonary effects of aerosolized machining fluids in mice and guinea pigs. *The Toxicologist* 10: 222 (1991) (Paper presented at the Society of Toxicology Meeting, Dallas, TX, 1991).
9. **Detwiler-Okabayashi, K; MM Schaper:** Acute respiratory effects from components of metalworking fluids in mice. *The Toxicologist* 14: 309 (1994) (Paper presented at the Soc. of Toxicology Meeting, Dallas, TX, 1994).
10. **Schaper, MM; KA Detwiler-Okabayashi, and SP Krystofiak:** Identification of irritants present in metalworking fluids. Paper presented at the American Industrial Hygiene Conference and Exposition, Anaheim, CA, 1994, Paper 142.
11. **Detwiler-Okabayashi, K; MM Schaper:** Evaluation of respiratory irritation of a metalworking fluid (MWF) vs. its components. *The Toxicologist* 15: 42 (1995) (Paper presented at the Society of Toxicology Meeting, Baltimore, MD, 1995).
12. **Krystofiak, S; KA Detwiler-Okabayashi, and MM Schaper:** Prediction of an occupational exposure limit for a metal-working fluid (MWF) on the basis of its individual components. Paper presented at the American Industrial Hygiene

- Conference and Exposition, Kansas City, MO, 1995, Paper 70.
13. **Schaper, MM; KA Detwiler-Okabayashi:** An approach for evaluating the respiratory irritation of mixtures: application to metal-working fluids. *Arch. Toxicol.*: In press (1995).
  14. **Krystofiak, SP; MM Schaper:** Prediction of an occupational exposure limit for a mixture on the basis of its components: application to metal-working fluids. *Am Ind Hyg Assoc J*: In press (1995).
  15. **Detwiler-Okabayashi, KA; MM Schaper:** Respiratory effects of synthetic metalworking fluid and its components. *Arch. Toxicol.*: In press (1995).
  16. **Alarie, Y:** Sensory irritation by airborne chemicals. *CRC Crit. Rev. Toxicol.* 2: 299-366 (1973).
  17. **American Society for Testing and Materials:** *Standard Test Method for Estimating Sensory Irritancy of Airborne Chemicals.* ASTM Designation E 981-84. Philadelphia, PA: American Society for Testing and Materials (1984).
  18. **Alarie, Y; JE Luo:** Sensory irritation by airborne chemicals: a basis to establish acceptable levels of exposure. In *Toxicology of the Nasal Passages*, ed. by C.S. Barrow. Washington, D.C.: Hemisphere Publishing Corporation, 1986. pp.91-100.
  19. **Ferguson, JS; MM Schaper, MF Stock, DA Weyel, and Y Alarie:** Sensory and pulmonary irritation with exposure to methyl isocyanate. *Toxicol. Appl. Pharmacol.* 82: 329-335 (1986).
  20. **Vijayaraghavan, R; MM Schaper, R Thompson, MF Stock, and Y Alarie:** Characteristic modifications of the breathing pattern of mice to evaluate the effects of airborne chemicals on the respiratory tract. *Arch. Toxicol.* 67: 478-490 (1993).
  21. **Vijayaraghavan, R; MM Schaper, R Thompson, MF Stock, LA Boylstein, JE Luo, and Y Alarie:** Computer-assisted recognition and quantitation of the effects of airborne chemicals acting at different areas of the respiratory tract in mice. *Arch. Toxicol.* 68: 490-499 (1994).
  22. **Boylstein, LA; SJ Anderson, RD Thompson, and Y Alarie:** Characterization of the effects of an airborne mixture of chemicals on the respiratory tract and smoothing polynomial spline analysis of the data. *Arch. Toxicol.*: In press (1995).
  23. **Schaper, MM:** Development of a database for sensory irritants and its use in establishing occupational exposure limits. *Am. Ind. Hyg. Assoc. J.* 54: 488-544 (1993).
  24. **Weyel, DA; BS Rodney, and Y Alarie:** Sensory irritation, pulmonary irritation, and acute lethality of a polymeric isocyanate and sensory irritation of 2,6-toluene diisocyanate. *Toxic. Appl. Pharmacol.* 64: 423-430 (1982).
  25. **Weyel, DA; RB Schaffer:** Pulmonary and sensory irritation of diphenylmethane-4-4'- and dicyclohexylmethane-4-4'-diisocyanate. *Toxicol. Appl. Pharmacol.* 77: 427-433 (1985).
  26. **Alarie, Y:** Toxicological evaluation of airborne chemical irritants and allergens using respiratory reflex reactions. In *Proceedings of the Inhalation Toxicology and Technology Symposium*, ed. by BKJ Leong. Ann Arbor, Michigan: Ann Arbor Science Publications, 1981. pp.207-231.

## Sensory Irritation Potential of Commercially Available Metalworking Fluids

Ann Ball and W. E. Lucke

Cincinnati Milacron Products Division  
P. O. Box 9013 Cincinnati, OH 45209

Seventeen commercially available metalworking fluids (two synthetic emulsions, two soluble oils, six semi-synthetics and seven synthetics) were tested for sensory irritation potential, as measured by RD<sub>50</sub> values, using the mouse bioassay with the following results:

a) The magnitudes of the RD<sub>50</sub> values are in general agreement with those reported by Schaper and Detwiler. The average RD<sub>50</sub> for the entire group was 319 mg/m<sup>3</sup> with a range of 126–953 mg/m<sup>3</sup>.

b) Two of the synthetic fluids could not be aerosolized sufficiently to achieve mist concentrations which would depress the breathing rates of the animals by 50%.

c) All fluids evoked sensory irritation immediately at the beginning of exposure, accompanied by a rapid decrease in respiratory frequency. As the length of exposure increased, sensory irritation persisted. For most fluids, evidence of pulmonary irritation was seen, particularly after 1-2 hours of exposure. After three hours, the predominant respiratory effect was pulmonary irritation for all but two fluids; a semi-synthetic and one of the synthetics which could not be aerosolized.

d) The animals did not return to a full recovery of normal respiratory frequency during the 20 minute postexposure observation period. However, the degree of recovery was inversely proportional to the exposure concentrations; animals exposed at lower concentrations showed greater degrees of recovery.

e) Comparison of RD<sub>50</sub> values by class of fluid suggested that the synthetics, as a group, were less irritating than the soluble oils, semi-synthetics or synthetic emulsions. However, when RD<sub>50</sub> values for ten fluids from an earlier study by

Schaper and Detwiler were combined with those of the present study, no significant differences were found between the soluble oils and the synthetic fluids. There appears to be as much variation within fluid classes as there is between the classes.

f) Replicate determinations were done for a semi-synthetic and a synthetic so that experimental error could be estimated. The standard error for the synthetic fluid was 29% of the mean while the standard error for the semi-synthetic was 18%.

g) A semi-synthetic fluid was submitted as a 10% dilution and as the concentrate. The correlation in RD<sub>50</sub> values for the pair was in good agreement, supporting the claim by Schaper and Detwiler that the animals are actually exposed to anhydrous fluid residues.

The fifteen RD<sub>50</sub> values which were measured were used to develop regression models using the compositions of the fluids as input variables. It was assumed that the irritation potential was related to the chemical composition of a fluid with *n* components by the relationship

$$RD_{50} = k_1c_1 + k_2c_2 + \dots + k_n c_n + C_0$$

where *k<sub>n</sub>* represents the irritation potential of the *n*th component and *c<sub>n</sub>* is the concentration of the *n*th component and *C<sub>0</sub>* would represent some level of irritation expected in the absence of any chemical exposure. Given the possibility that two or more components have the potential to interact with one another through salt formation or other reaction, the model should also allow for possible second order terms of the form *k<sub>xy</sub>c<sub>x</sub>c<sub>y</sub>*. Since a higher value of RD<sub>50</sub> indicates that a material must be present at a higher level to cause a response, a positive value for a given *k<sub>n</sub>* indicates that substance *n* reduces the irritation potential of a mixture in which it is present.

The components evaluated for inclusion in this model were either discrete chemicals, classes of chemicals or a collective property of the fluid:

- Total Base Number—A measure of the fluid alkalinity (TBN)
- Ethanolamine—(MEA)
- Triethanolamine—(TEA)
- Aminomethylpropanol—(AMP)
- Sodium tolyltriazole—(TT)
- Petroleum sulfonates—(Sulfonates)
- Fatty Amides—(Amide)
- Tall oil fatty acids —(TOFA)
- Fatty esters —(Ester)
- Short chain mono- and dicarboxylic acids C<sub>9</sub>–C<sub>12</sub> —(Acid)
- Formaldehyde release biocides, expressed as equivalent formaldehyde content—(“H<sub>2</sub>CO”)

Multiple stepwise regression of RD<sub>50</sub> against the composition data yields a model of the form

$$RD_{50} = 194.6 + 5.8 \times [MEA] \times [Acid] + 2.6 \times [TEA] \times [Acid]$$

with R<sup>2</sup> = 0.94.

Note that both k<sub>n</sub>'s are positive, indicating that the effect of both factors is to decrease the irritation potential of the fluids. The model predicts that the fluids have an inherent irritation potential that cannot be assigned to the presence of one or more components, but which can be reduced by incorporating both amines and acids. While it can be rationalized that neutralizing acids with amines would make each less irritating, and the data are well described statistically by the model, the failure to find an assignable cause for irritation is a major flaw in this model. Further, the failure to predict the known irritancy of the short chain acids makes the model suspect.

Most of the variability in the RD<sub>50</sub> values comes from the relatively high values of the

synthetic fluids. In fact, if the regression is done on the synthetics alone; the resulting model is not significantly different from that for the full data set. A regression on the data from only the semi-synthetics yields a model of the form

$$RD_{50} = 302.7 - 20.1 \times [Amide]$$

with R<sup>2</sup> = 0.787 (98% confidence). In this model, the fatty amides are identified as a source of irritation (k<sub>n</sub> < 0).

An alternate model was developed by using the raw respiratory rate reductions observed when the mice were exposed at varying mist levels as the output variable. The response (RD) in this case becomes greater as irritation increases; positive values of k<sub>n</sub> indicate that component *n* is irritating. Since all the data points are used, including those from the two fluids for which the RD<sub>50</sub> could not be estimated, the data set now includes 101 points. The resulting regression yields

$$RD = 24.7 + 0.49 \times [Sulfonates] + 0.14 \times [MEA] + 1.72 \times [Amides] + 0.43 \times [TOFA]$$

and R<sup>2</sup> = 0.57 (99.9% confidence).

All the factors in this model are predicted to be causes of irritation. Three of them are surface active; the fourth is alkaline. The model makes sense from a chemical standpoint and identifies the fatty amides as the most potent irritant, in agreement with the RD<sub>50</sub> model for the semi-synthetics only.

Stepwise regression of the RD<sub>50</sub> data, excluding the synthetic fluids, gives the expression

$$RD_{50} = 558.1 - 47.0 \times [Amide] - 18.5 \times [Sulfonate] - 18.4 \times [TOFA]$$

with R<sup>2</sup> = 0.42 (90% confidence).

This model agrees with the Respiratory Depression model in the relative magnitudes of the effects of the surface active agents, but does not identify MEA as an irritant.

The results of this study suggest

- Surface active substances, and possibly

amines, contribute most to the sensory irritation potential of metalworking fluids.

- Further work should focus on using a better experimental design to reduce the effects of uncontrolled factors on variances.
- Use of individual responses at each exposure level, rather than the value collected from these responses, would be expected to increase

the power of future studies.

The mouse bioassay fails to identify the short chain acids as irritants, in contradiction to shop floor experience. This may reflect a difference between the mouse and human or the presence of a different mechanism of irritation. High  $RD_{50}$  values for synthetic fluids containing these acids do not reflect the true irritation potential of these fluids.

## Respiratory Health Effects of Machining Fluids in Laboratory Animals

Peter S. Thorne and Jeannine A. DeKoster

The University of Iowa, Department of Preventive Medicine  
& Environmental Health, Iowa City, IA 52242-5000

### ABSTRACT

Acute respiratory health effects of inhalation exposure to neat and in-use metalworking fluids (MWF), and specific contaminants of in-use MWF were studied using established guinea pig and mouse inhalation toxicology models to evaluate the potency of characterized MWF in terms of their effects on breathing, pulmonary hyper-sensitivity, and lung inflammation. Inhalation exposure of guinea pigs to MWF caused a dose-dependent increase in respiratory rate and decrease in breathing volume that was used to quantify MWF potency. In all cases, the in-use MWF were more toxic than their corresponding neat MWF. Adjusting the pH of the neat MWF or spiking the fluid with active cultures of *Pseudomonas pseudoalcaligenes* (the predominant Gram negative bacterium in many of the MWF samples) did not increase the potency to the level of the in-use MWF. From these data significant predictors of respiratory responses were whether the fluid was neat or in-use ( $p=0.0001$ ), the exposure concentration (dose) ( $p=0.022$ ), the particular MWF formulation tested ( $p=0.031$ ), and the particular in-use fluid sample tested ( $p=0.032$ ). Inhalation exposures to biocides used in the MWF resulted in dose-dependent decreases in respiratory rate consistent with sensory irritation responses.

Additional guinea pig studies were conducted to investigate the biologic responses in the lung to MWF inhalation. These studies revealed that there was significant inflammation resulting from a 3 hr exposure to MWF aerosol at 50 to 77 mg/m<sup>3</sup>. This inflammatory response was marked by a change in the bronchoalveolar lavage (BAL) fluid from 3% neutrophils in control animals to 60 - 79% in the MWF-exposed guinea pigs. Total cells in the lung lavage increased from 0.4 x 10<sup>6</sup> cells/ml in controls to between 7.7 x 10<sup>6</sup> and 11.3 x 10<sup>6</sup> cells/ml in the exposed groups. The in-use MWF used for these studies ranged in endotoxin

concentration from 280 to 1.7 x 10<sup>5</sup> EU/ml from Gm- bacteria contaminating the MWF. To investigate the role of endotoxin in the acute toxicity of the MWF, studies were carried out in normal mice (SEN) that revealed a dose dependent 10,000-fold increase in neutrophils in the lavage following inhalation exposure to in-use MWF. This recruitment of neutrophils was in response to a 100-fold increased concentration of the cytokines: interleukin-6 (IL-6) and tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) in the lung induced by the inhaled MWF. A smaller increase was seen in the lavage concentration of interleukin-1 $\alpha$  (IL-1 $\alpha$ ). This inflammatory response was not observed with exposure to neat MWF or with sham exposure. A different strain of mice (RES) with a genetic defect that rendered them hypo-responsive to endotoxin were tested in parallel with the mice just described and demonstrated virtually no response to the inhaled MWF in terms of total cells, neutrophils, IL-6, or TNF $\alpha$ . Removal of micro-organisms from the MWF by filtration of the in-use MWF did not change the responses observed in either strain of mouse. These studies and in-plant exposure assessment reported elsewhere suggest that lung inflammation may be an important outcome from exposure to in-use MWF and that endotoxin is a toxicant of importance. Studies in both guinea pigs and mice demonstrated the importance of MWF-induced lung inflammation in the pathophysiology of acute respiratory responses to machining fluids.

### INTRODUCTION

Machining fluids are used to cool, flush, and lubricate metal parts and machining tools during machining operations. Soluble oil and semi-synthetic MWF predominate in automotive manufacturing and are commonly mixtures of 20 or more ingredients in an alkaline solution with 2

to 20 % emulsified oil.<sup>(1)</sup> They contain few contaminants when introduced to the MWF distribution systems (as "neat" fluids), but with use become contaminated with debris, metals, thermal breakdown products, microorganisms, and microbial products. Microorganisms colonize MWF in suspension or as biofilms and act as sources of a variety of microbial products and toxins. Biocides are antimicrobial chemicals that are added to in-use MWF to control bacterial and fungal growth. With time, in-use MWF become complex chemical and microbial mixtures that yield vapors and aerosols when flushed onto bar stock, forgings, casts, and machining tools.

There have been several animal toxicology studies reported that have yielded differing conclusions as to the relative toxicity of neat versus in-use MWF.<sup>(2,3)</sup> Further, few toxicology studies reported to date have explored the pathophysiology of the responses to inhaled in-use MWF. The purpose of this study was to characterize acute pulmonary responses to inhaled neat and in-use MWF and identify potential causative agents.

## METHODS

### Animal Exposure Protocols

Experimental protocols were approved by the Institutional Animal Care and Use Review Committee and housed in AAALAC accredited facilities. Virus-free, male English Shorthair guinea pigs, *Cavia cobaya* (Sasco, Omaha, NE) were received at 200-250 g. Male *Mus musculus* (RES, strain C3H/HeJ and SEN, strain C3H/HeBFEJ) were purchased (Jackson Lab., Bar Harbor, ME) at 5 wks of age. Both species were housed 4 to a cage and provided with food and water *ad libitum*. Guinea pig inhalation exposures were performed in a whole-body plethysmograph exposure system described previously.<sup>(4,5)</sup> Dose-response studies for acute changes in pulmonary function and tests for hyper-sensitivity were performed in guinea pigs. For both, baseline measurements of respiratory rate and volume as well as flow-volume loops were measured the day before the first exposure. Dose-response studies began with 3 hr baseline monitoring, followed by 3

hr exposure and 2 hr post-monitoring. Guinea pigs were removed from the plethysmographs and flow-volume loops were taken at 5 hours post-exposure. For murine exposure studies, groups of 12 mice (6 RES and 6 SEN) were exposed in whole body exposure chambers for 4 hr. Aerosols of new and in-use MWF and specific toxicants of MWF were generated using a Pitt No. 1 nebulizer operated at a pressure head of 101.3 kPa gauge. Concentrations were controlled by adjusting the syringe pump (Harvard Apparatus, S. Natick, MA) feed rate to the nebulizer or the chamber exhaust rate.<sup>(4,5)</sup> A magnetically coupled rotor was used to achieve proper distribution of aerosol within exposure chambers.

### Aerosols, Endotoxins and Bioaerosols

MWF aerosol concentrations in the exposure chambers were determined gravimetrically using EPM-2000 filters (Whatman International, Maidstone, England) at 2.0 L/min in a closed-face configuration. Filters were desiccated and then weighed on a Mettler MT-5 microbalance (Mettler Instr. Corp., Hightstown, NJ). Mass particle size distributions were determined using a 7-stage cascade impactors (Mercer, In-Tox, Albuquerque, NM) operated at 2.0 L/min. Count particle size distributions were measured using an APS-33B (TSI, Inc., St. Paul, MN) time-of-flight aerodynamic particle sizer.

Endotoxin analysis was performed on air sampling filters following gravimetric analysis and used the chromogenic *Limulus* amoebocyte lysate assay (BioWhittaker, QCL-1000, Walkersville, MD). Filters were extracted into pyrogen-free water (30 ml) with 30 min heating at 68°C.<sup>(2)</sup> Serial dilutions of the extract from filters along with endotoxin standards were prepared using sterile pyrogen-free water in borosilicate glass tubes which had been heat treated for at least 4 hr at 200°C. Absorbance was measured at 405 nm in a temperature controlled microplate reader (BT2000, Bio-Tek Instruments, Palo Alto, CA). Change in absorbance relative to the assay reagent blank was determined and endotoxin concentration calculated from a standard curve accounting

for filter elution volume and eluate dilution. The standard curve ranged from 0.1 to 1.0 Endotoxin Units (EU) of EC-5 standard endotoxin (10 EU= 1 ng).

Viable bioaerosol concentrations in exposure chambers were determined with 2-stage Andersen Microbial Samplers (Graesby-Andersen, Atlanta, GA) operated at 28.3 L/min<sup>(6)</sup> and all-glass impingers (AGI-30, Ace Glass Inc., Vineland, NJ) with 12.5 L/min airflow.<sup>(7)</sup> Culture media were R2A for mesophilic bacteria, EMB for Gm-mesophilic bacteria, and MEA for fungi. All plates were incubated for 5 days at 23 ± 2°C. The AGI-30 sampling media was a 1% peptone solution.<sup>(7)</sup> Total microorganisms were determined using the fluorescence microscopy Nuclepore filtration / elution method (FM/NFE).<sup>(7,8)</sup>

### Pulmonary Function Monitoring

Respiratory frequency and plethysmographic pressure changes were determined using differential pressure transducers (Gaeltec 8T-2, MMI, Hackensack, NJ) and signal amplifiers (Carrier Amps 20-4615-35, Gould Inc., Valley View, OH). Amplifier outputs were digitized (Keithley Inst. Inc., Cleveland, OH), displayed, and stored in real time. The amplitude of the pressure changes was calibrated using a rodent respirator (Harvard Apparatus, S. Natick, MA). Calibrated pressure changes were expressed in volume-equivalent units and approximated breathing volumes.<sup>(5)</sup> Flow-volume loops were obtained using a head only plethysmograph as described previously.<sup>(5)</sup> In this system, inspiratory and expiratory flows were determined using a Fleisch pneumotacograph (OEM Medical Inc., Richmond, VA) connected to a pressure transducer (Gaeltec 8T-2) and monitored as above. Calibrated flow signals were integrated digitally to yield volume signals from which flow-volume loops were plotted.

### Necropsy and Analysis of BAL Fluid

For necropsy, guinea pigs were first injected i.p. with 0.05 ml atropine (0.4 mg/ml, Elkins-Sinn, Inc., Cherry Hill, NJ) followed 15 minutes later by

an intraperitoneal injection of pentobarbital Na, (75 mg/kg, Abbott Labs, N. Chicago, IL). Mice were euthanized by cervical dislocation. The diaphragm was incised from the peritoneal space via a mid saggital incision and the animals were exsanguinated from the heart. The trachea was then exposed and canulated with PE tubing. Guinea pig lungs were lavaged three times with 5 ml of 0.9% sterile saline and the recovered lavage fluid was pooled. Mouse lungs were lavaged 6 times with 1 ml of saline. The lungs of some animals were instilled with Karnofsky's fixative and stained for histo-pathological study.

BAL fluids were centrifuged at 2300 rpm for 5 min and supernatants were decanted and stored at -70°C to await cytokine analysis. Pellets were resuspended in HBSS and cells were counted using a hemacytometer. An aliquot of this solution was cytocentrifuged (StatSpin, Norwood, MA) onto a glass slide and stained (Diff Quick, Baxter, McGaw Park, IL). Cells were counted differentiating between macrophages, neutrophils, lymphocytes, eosinophils, and airway epithelial cells. Murine cytokines in the BAL fluid were assayed by enzyme-linked immunosorbent assay (ELISA) kits and included TNF $\alpha$  and IL-1 $\alpha$  (Genzyme, Cambridge, MA) and IL-6 (Endogen, Inc., Cambridge, MA). None of these cytokines demonstrated cross reactivity with the other or with GM-CSF,  $\gamma$ IFN, IL-2, IL-3, IL-4, IL-5, or IL-7.

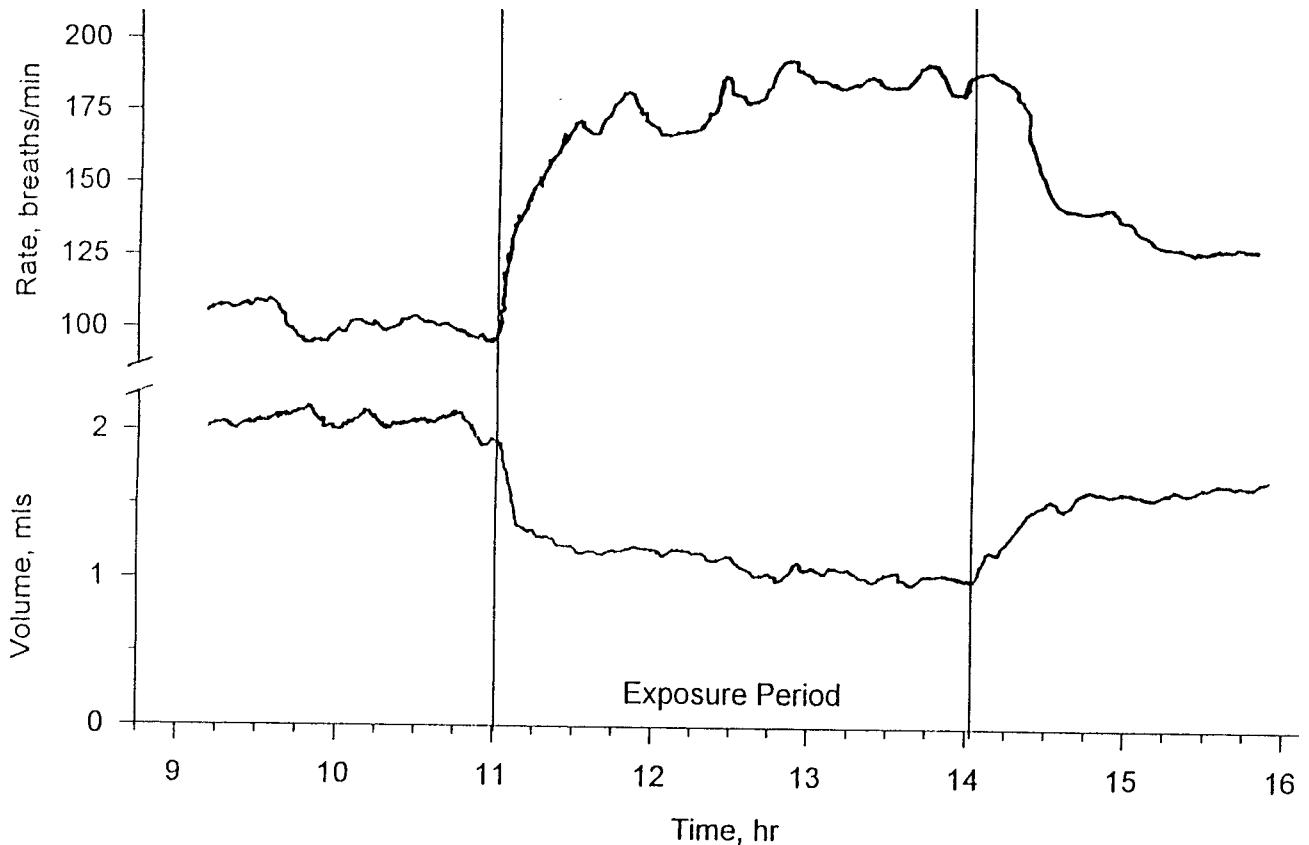
## RESULTS

### Guinea Pig Studies

Groups of guinea pigs were exposed to MWF aerosols in whole body plethysmographs with continuous monitoring of their breathing. MWF aerosol size distributions were unimodal and poly-disperse with a mass median aerodynamic diameter of 0.83  $\mu$ m ( $\sigma_g = 2.0$ ). MWF exposures generally induced an increase in respiratory rate and a decrease in respiratory volume that returned to near baseline by 2 hr after exposure. Figure 1 is a sample tracing of the pulmonary response of a guinea pig exposed to a neat MWF for 3 hr at 165 mg/m<sup>3</sup>. The upper curve illustrates a sudden increase in respiratory rate at the start of exposure

from the baseline value of 100 breaths/min up to 190 breaths/min. By 2 hr post-exposure the rate had fallen to 125 breaths/min. The respiratory volume fell during the exposure from 2.0 ml to about 1.1 ml and then showed recovery in the post-

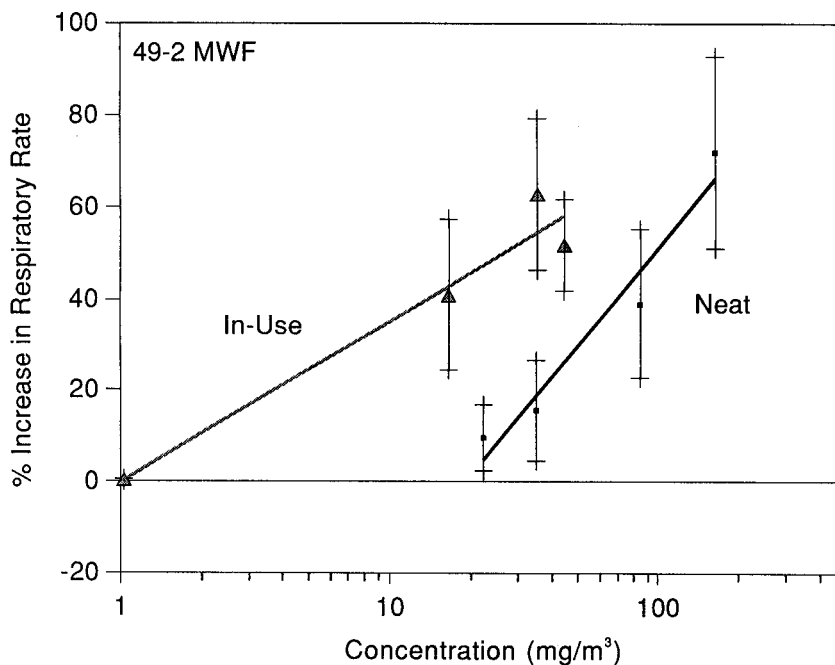
exposure period (lower curve). Data such as that shown in Figure 1 from groups of guinea pigs (N=4-8) exposed at differing concentrations of MWF samples formed the basis for establishing dose-response relationships.



**Figure 1.** Plethysmographic tracings of the pulmonary response of a guinea pig exposed to neat MWF for 3 hours at  $165 \text{ mg/m}^3$ . Respiratory rate increased dramatically at the start of exposure while respiratory volume fell during the exposure and then recovered in the post-exposure period.

Figure 2 illustrates dose-response curves for a neat MWF diluted to the in-use concentration (right) and the corresponding in-use MWF (left). The relative toxicity of these MWF was quantified by establishing the concentration that was predicted to yield a 50% increase in respiratory rate ( $RC_{50i}$ ) or a 35% decrease in respiratory volume ( $VC_{35d}$ ) during the exposure. For the neat MWF illustrated in Figure 2, the  $RC_{50i}$  was  $94 \text{ mg/m}^3$ , considerably higher than the in-use MWF value of  $26 \text{ mg/m}^3$ . These and other values are shown in Table 1 for 3 MWF and indicate that the in-use

MWF were all more potent respiratory toxicants than the corresponding neat fluid. In addition, the  $VC_{35d}$  for these fluids were very similar to the  $RC_{50i}$ . Analysis of these data showed that whether the fluid was neat or in-use ( $p=0.0001$ ), exposure concentration ( $p=0.022$ ), and the fluid formulation tested ( $p=0.031$ ) were all significant predictors of respiratory responses. Histopathology studies and analysis of BAL fluid from guinea pigs exposed to in-use MWF demonstrated a dose dependent recruitment of neutrophils to the lungs, a hallmark of inflammation.



**Figure 2.** The dose-response curve in guinea pigs for a soluble oil in-use and neat machining fluid is shown for respiratory rate and illustrates increased potency of the in-use fluid.

**Table 1.** Guinea Pig Pulmonary Responses to Neat and In-Use Metal Working Fluids

Metal Working Fluid Sample	RC <sub>50i</sub> Aerosol Conc <sup>a</sup> for 50% Increase in Respiratory Rate, mg/m <sup>3</sup>		VC <sub>35d</sub> Aerosol Conc <sup>a</sup> for 35% Decrease in Respiratory Volume, mg/m <sup>3</sup>	
	Neat	In-Use	Neat	In-Use
4 9-1	>150 <sup>b</sup>	22	>150 <sup>b</sup>	23
4 9-2	94	26	105	25
4 9-3	308	60	348	60

<sup>a</sup> MWF exposures were determined gravimetrically following filter desiccation

<sup>b</sup> No significant change in respiratory rate or volume was seen with exposures up to 150 mg/m<sup>3</sup> for this neat MWF

Inhalation experiments similar to those described above were performed to assess the toxicity of formaldehyde and isothiazolines. Whereas the MWF caused an increase in

respiratory rate during inhalation exposure both the formaldehyde and isothiazolines produced a dramatic decrease in the respiratory rate within the first 20 minutes of the initiation of exposure indicative of sensory and pulmonary irritation. The depressed respiratory rate continued through the exposure. The concentration that induced a 50% decrease in respiratory rate during exposure,  $RC_{50d}$ , was 20.8 mg/m<sup>3</sup> for formaldehyde and 8.1 mg/m<sup>3</sup> for a commercial mixture of 2-methylisothiazolin-3-one and 5-chloro-2-methylisothiazolin-3-one.

The respiratory effects of Gram negative bacteria in the in-use MWF were assessed by inoculating neat MWF with active cultures of *Pseudomonas pseudoalcaligenes* (ATCC# 17443) to yield 10<sup>6</sup> CFU/ml. This bacterium was chosen because it was the microorganism found most

commonly in the metal working fluids. No significant differences were seen in respiratory responses between the exposures with and without the *P. pseudoalcaligenes*.

### Mouse Studies

Inhalation studies were carried out using a 2 strain mouse model that has been used extensively to investigate grain dust induced lung inflammation.<sup>(9,10)</sup> Endotoxin resistant mice (RES) and mice with normal endotoxin sensitivity (SEN) were exposed by inhalation to saline (sham exposure), to neat MWF, to the corresponding in-use fluid at varying concentrations, and to the in-use fluid after filter sterilization (0.2 µm) to remove microorganisms and debris. The results of these experiments are summarized in Table 2.

**Table 2.** Bronchoalveolar lavage fluid responses for endotoxin sensitive (SEN) and resistant (RES) mice exposed to 49-3B neat and in-use metal working fluid at various concentrations<sup>a</sup>

Group	Endotoxin, µg/m <sup>3</sup>	Strain	% MΦ	% PMN	TNFα	IL-6
Sham	< 0.02	SEN	85.8	1.5	17 <sup>b</sup>	11 <sup>b</sup>
		RES	97.8	0.3	31	11
Neat	< 0.02	SEN	80.5	0.3	17	11
		RES	88.8	0.7	17	12
In-Use	0.27	SEN	75.2	4.8	--	--
		RES	83.4	1.8	--	--
In-Use	0.43	SEN	42.3	49.0	101	17
		RES	89.8**	0.8**	17*	11
In-Use	3.85	SEN	1.7	96.7	3160	1340
		RES	77.7**	5.2**	55**	11**
In-Use	59.1	SEN	2.7	92.8	1820	1090
		RES	77.3**	3.3**	128**	14**
In-Use	6.52	SEN	4.3	94.8	2690	2190
		RES	93.2**	0.5**	44**	11**

<sup>a</sup> In-use MWF exposures expressed in µg/m<sup>3</sup> of airborne endotoxin (note 1 µg/m<sup>3</sup> = 10<sup>4</sup> EU/m<sup>3</sup>)

<sup>b</sup> Lower Limits of detection for cytokines: IL-6=11 pg/ml, TNF-α=17 pg/ml

\* Difference between RES and SEN mice at p<0.05

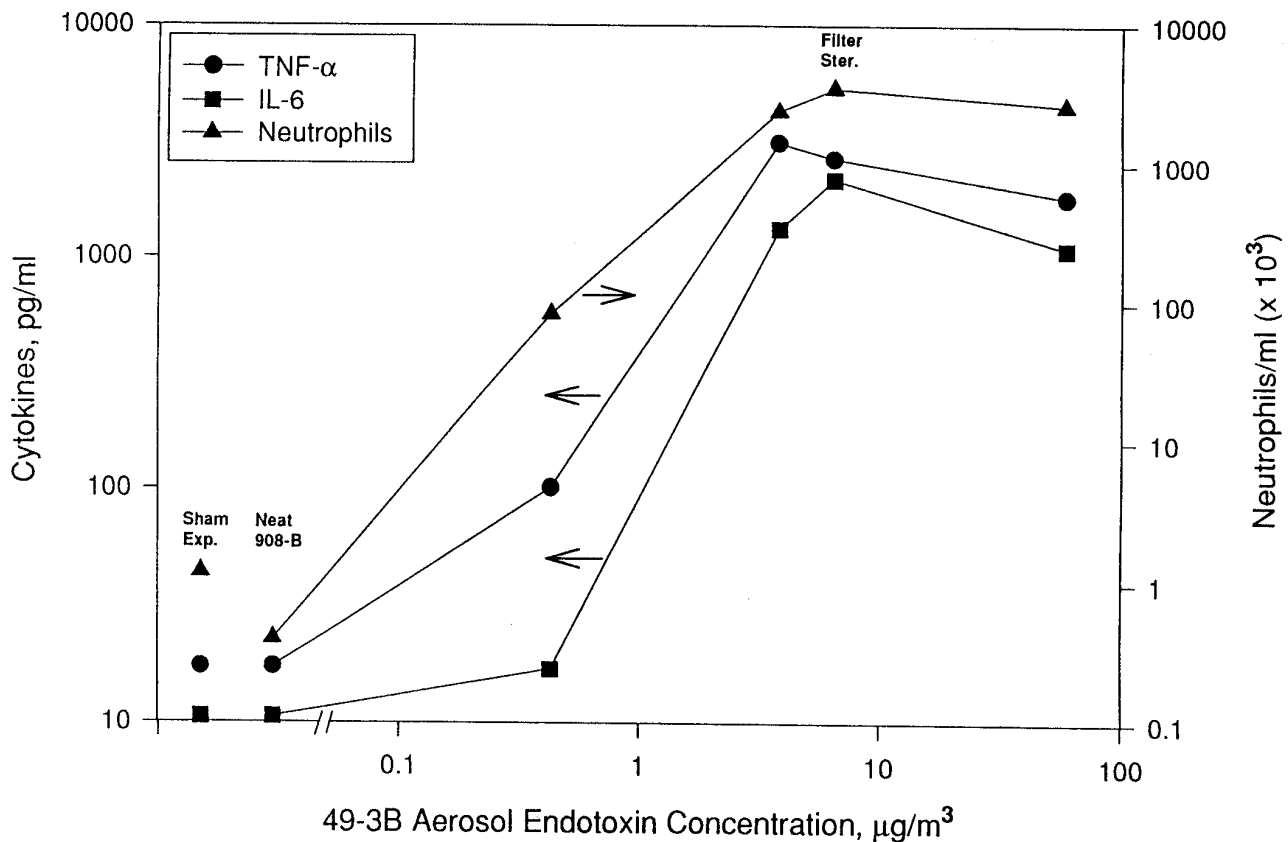
\*\* Difference between RES and SEN mice at p<0.01

No differences in total cells (not shown), macrophages (MΦ), or neutrophils (PMN) in the BAL fluid between the RES and SEN mice were

observed for the sham-exposed mice, those exposed to the neat MWF, or those exposed to the lowest in-use concentration. As the endotoxin

concentration increased from 0.27 to 0.43, 3.9, and 59  $\mu\text{g}/\text{m}^3$ , the total cell counts and the %PMN in the BAL fluid increased highly significantly ( $p < 0.01$ ) in the SEN mice. The endotoxin RES

mice showed a blunted response that was highly significantly different ( $p < 0.01$ ) from the SEN mice. This is plotted in Figure 3.



**Figure 3.** The cytokine and neutrophilic response to inhalation of in-use machining fluid. TNF- $\alpha$ , IL-6 and neutrophil concentrations were minimal for mice exposed to neat fluid or sham exposed, but were increased markedly with exposure to increasing concentrations of in-use MWF expressed as airborne endotoxin. Filter sterilization of the fluid did not alter the response.

This effect was also seen in the mice exposed to filter-sterilized in-use MWF ( $6.52 \mu\text{g}/\text{m}^3$ ), suggesting that the inflammatory response was due to the soluble factors and not the intact organisms or other particles. Data from other experiments<sup>(9)</sup> in which mice were exposed to solutions of endotoxin (from *E. coli*) in saline revealed very similar responses.

Cytokine assays for murine TNF- $\alpha$ , IL-1 $\alpha$ , IL-6 were performed on BAL fluid from some groups of mice exposed to MWF. IL-1 $\alpha$  was not increased appreciably 5 hr after the start of exposure. TNF- $\alpha$  and IL-6 were both below

detection limits for mice exposed to neat MWF or sham exposed. However, both cytokines increased markedly in the SEN mice with exposure to increasing concentrations of in-use MWF expressed as airborne endotoxin (Figure 3). Filter sterilization of the MWF did not alter the response. Conversely, the response for the RES mice to the in-use MWF was minimal for TNF- $\alpha$  and non-existent for IL-6 (Table 2). TNF- $\alpha$  and especially IL-6 showed a highly significant difference between the SEN and RES mice exposed to the higher endotoxin concentrations. This finding parallels the endotoxin dose dependent neutrophil

response in guinea pigs and mice and indicates the inflammatory nature of the inhaled in-use MWF at these concentrations.

## CONCLUSIONS

Studies in guinea pigs and mice demonstrated the importance of MWF-induced lung inflammation in the pathophysiology of acute respiratory responses to machining fluids. Differential responses in endotoxin sensitive and resistant mice and endotoxin dose-dependent responses for proinflammatory cytokines and lung neutrophils provide strong evidence that endotoxin in machining plants could be a significant hazard. Our findings (presented elsewhere in this volume) that airborne endotoxin is present in automotive machining plants at concentrations exceeding human pulmonary response thresholds along with these toxicology studies, point to the importance of carefully controlling microbial growth and endotoxin formation in MWF in order to reduce acute respiratory health effects among machinists.

## ACKNOWLEDGMENTS

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## REFERENCES

1. **Sprince N; PS Thorne, and MR Cullen:** Oils and related petroleum derivatives. In: *Textbook of Clinical Occupational and Environmental Medicine* (L Rosenstock and MR Cullen, eds.) W.B. Saunders, Co., Orlando, FL, Chapter 34:814-825, 1994.
2. **Gordon, T:** Acute respiratory effects of

endotoxin-contaminated machining fluid aerosols in guinea pigs. *Fundamental and Applied Toxicology* 19:117-123 (1992).

3. **Schaper, M; K Detwiler.** Evaluation of the acute respiratory effects of aerosolized machining fluids in mice. *Fundamental and Applied Toxicology* 16:309-319 (1991).
4. **Thorne, PS; JA Hillebrand, C Magreni, EJ Riley, and MH Karol:** Experimental sensitization to subtilisin: I. Production of immediate- and late-onset pulmonary reactions. *Toxicol. and Appl. Pharmacol.* 86:122-123 (1986).
5. **Thorne, PS; MH Karol:** Assessment of airway reactivity in guinea pigs: comparison of methods employing whole-body plethysmography. *Toxicology* 52:141-163 (1988).
6. **DeKoster JA; PS Thorne:** Bioaerosol concentrations in non-complaint, complaint and intervention homes in the midwest. *Am. Ind. Hyg. Assoc. J.* 56:573-580, 1995.
7. **Thorne PS; JL Lange, PD Bloebaum, and GJ Kullman:** Bioaerosol sampling in field studies: Can samples be express mailed? *Am. Ind. Hyg. Assoc. J.* 55:1072-1079 (1994).
8. **Thorne, PS; MS Kiekhaefer, P Whitten, and K Donham:** Comparison of bioaerosol sampling methods in barns housing swine. *Appl. Environ. Microbiol.* 58:2543-2551 (1992).
9. **Schwartz DA; PS Thorne, PJ Jagielo, GE White, SA Bleuer, and KL Frees:** Endotoxin responsiveness and grain dust-induced inflammation in the lower respiratory tract. *J Am Physiol* 267 (Lung Cell Mol Physiol 11): L609-L617, 1994.
10. **Jagiello PJ; PS Thorne, JL Watt, KL Frees, TJ Quinn, and DA Schwartz:** Grain dust & endotoxin inhalation produce similar physiologic and inflammatory responses in normal subjects, 1995 (in press).
11. **UAW-GM National Joint Committee on Health and Safety.** Research agreement between UAW-GM and The University of Iowa. pg. 5, 1991.

## Acute Effects of Metal Working Fluids in a Respiratory Inflammation Model

Donald K. Milton, Joseph D. Brain, and Dianne D. Rees  
Department of Environmental Health, Harvard School of Public Health

### ABSTRACT

The relative ability of used and unused metal working fluids (MWF) to elicit lung injury and inflammation was studied in hamsters exposed by intratracheal instillation. Samples were collected to represent the major soluble MWF from each of two plants participating in UAW-GM sponsored epidemiologic studies. Concurrent with collection of used fluids, samples of the neat fluids were also obtained. The major constituents of the two soluble fluids, TMS-9 and T-Kool 145A are different. TMS-9 is petroleum oil based and T-Kool is primarily hydrotreated heavy naphthenic distillate. Both fluids contain petroleum sulfonates, although the Material Safety Data Sheets (MSDSs) indicated that the sulfonates differed and that TMS-9 contains more sulfonate (15-20%) than T-Kool (5-15%). Both also contain fatty acid derivatives but not the same ones, and the remaining minor constituents are different.

### INTRODUCTION

As reported elsewhere in this Symposium, the epidemiologic studies at the two plants found significantly different exposure levels, found more frequent acute changes in pulmonary function in the plant having higher particulate and endotoxin levels. This toxicologic investigation examined whether any of the differences between the plants can be attributed to differences in the toxic potency or the endotoxin content of the fluids themselves.

Fluid composition based on the MSDS provided to GM by the fluid manufacturers was: TMS-9 is 70-80% petroleum oil (Chemical Abstracts Service number [CAS#] 64741-96-4), 15-20% petroleum sulfonates (CAS# 68608-24-2), 3-8% chlorinated paraffin (CAS# 68920-70-7), 1-5% fatty acid derivatives (CAS# 8016-28-2), 1-5% ethyl-hydroxymethyl-oleyl-oxazoline (CAS#

68140-98-7), and 0.5-3% non-phenolic biocide (CAS# 65330-59-8). T-Kool 145A is 75% severely hydrotreated heavy naphthenic distillate (CAS# 64752-52-5), 5-15% petroleum sulfonate (CAS# 68608-26-4), 2-5% tall oil fatty acid sodium salts (CAS# 61790-45-2), <3% Sulfated vegetable oil (CAS# 68187-76-8), <2.5% alcohol's, C<sub>12-18</sub> ethoxylated and propoxylated (CAS# 69227-21-0), <2% dipropylene glycol (CAS# 25265-71-8), <2% fatty acid esters (CAS# 84501-87-1), and <1% pine oil (CAS# 8002-09-3).

A semi-synthetic fluid, TRIM-CE was also used in one of the plants. According to the MSDS, it contained between 1 and 10% severely hydrotreated petroleum oil (CAS# 8002-05-9), petroleum sulfonate (CAS#61789-85-3), chlorinated alkene polymer (CAS#68410-99-1), and triethanolamine borate (CAS#68512-53-8). It contained less than 1% by volume of several surfactants, a silicone defoamer, dye and tolytriazole, isothiazolin, and sodium pyrrithione. The remainder was water. A sample of unused TRIM CE was tested at the same time as the used and unused samples of the soluble fluids.

### METHOD

Animal exposures were performed by intratracheal instillation of MWF diluted with saline. Prior to instillation, each of the used fluids was adjusted so that it contained the same oil content 3.7%. Neat fluids were also diluted to 3.7% oil content with endotoxin free water and the resulting unused fluids contained no detectable endotoxin. Each of the unused and used fluids was then diluted in endotoxin free saline prior to instillation. Animals were instilled with 0.25  $\mu$ l, 1.25  $\mu$ l, 6.25  $\mu$ l, or 31.25  $\mu$ l/100 gm body weight in a volume of 150  $\mu$ l/100 gm. At the highest dose, the used TMS-9 instillate contained 149,000 Endotoxin Units (EU, referenced to EC5) /ml resulting in instillation of 22350 EU/100 gram/

animal. The used T-Kool instillate contained 190,000 EU/ml or 28500 EU/100 gram/animal.

Twenty-four hours after instillation with MWF, animals were sacrificed and their lungs lavaged with phosphate-buffered saline. The bronchoalveolar lavage fluid (BAL) was then analyzed for indicators of pulmonary injury or inflammation in response to the MWF. The parameters measured included lactate dehydrogenase (cell death), myeloperoxidase (neutrophil degranulation or death), albumin (epithelial permeability), hemoglobin (hemorrhage), and white blood cells numbers (inflammation).

To test the effect of endotoxin concentration on fluid toxicity, endotoxin was both added to and removed from one of the test fluids. Gram negative bacteria (GNB, *Serratia liquefaciens*) were cultured, washed, autoclaved and spiked into unused TMS-9. LPS neutralizing protein (LNP) from *Limulus* was used to reduce the endotoxin concentration in used TMS-9 prior to instillation. Unused TMS-9 spiked with GNB contained 252,000 EU/ml as prepared for instillation (37800 EU/100 gram/animal). Used TMS-9 treated with LNP contained 25,400 to 41,300 EU/ml (3810 to 6195 EU/100 gm) a 72% to 83% reduction in endotoxin dose.

## RESULTS

Instilled MWF caused both injury and inflammation in the hamster lungs. All measured parameters, except for myeloperoxidase, increased with increasing dose of each of the unused or used MWF. A pronounced increase in BAL white cell numbers was observed, reflecting a large influx of neutrophils into the lungs. While all the MWF studied produced a toxic response, there were measurable differences among them. The three unused MWF produced similar pulmonary responses. Exposure to the used MWF resulted in greater toxicity than exposure to the unused fluids. The used TMS-9 produced the largest responses of the four MWF. For example, BAL neutrophil numbers following exposure to the fluids were (mean  $\pm$ SE) 49.3  $\pm$ 4.9, 57.2  $\pm$ 13.6, 73.8,  $\pm$ 12.7, 134.9  $\pm$ 41.6 millions/animal and BAL albumin

levels were 1.31  $\pm$ 0.17, 1.11  $\pm$ 0.29, 2.89  $\pm$ 1.25, and 6.04  $\pm$ 1.89 mg/ml for unused TMS-9, unused T-Kool, used T-Kool and used TMS-9, respectively.

When animals were exposed to unused TMS-9 spiked with GNB, BAL albumin levels (4.5+0.77 mg/ml) and neutrophil numbers (102.9+11.2 millions/animal) rose to levels close to those for used TMS-9. Lactate dehydrogenase levels were unchanged. These results suggest that endotoxin in combination with unused TMS-9 produces pulmonary responses similar to those of used TMS-9. By contrast, used TMS-9 from which endotoxin had been largely removed did not produce responses identical to those for unused TMS-9. BAL albumin (0.19 mg/ml) and lactate dehydrogenase levels (64.3 mU/ml) were both lower after LNP treatment than levels after unused TMS-9. However, BAL neutrophil numbers were unchanged by LPN treatment. These results suggest that LPN treatment of used TMS-9 may remove toxic components other than endotoxin.

## CONCLUSIONS

In general, we found that used soluble MWFs had a greater ability to elicit lung injury and inflammation than unused samples, particularly at higher doses. The used samples were contaminated with gram negative bacteria and we found significant endotoxin in them. When unused samples were spiked with gram negative bacteria, we found a two-fold or more increase in epithelial permeability (albumin) and inflammation (neutrophils); the response of the spiked sample was similar to that of the used fluid. We conclude that the three unused MWFs had similar potency for eliciting inflammation and lung injury in this model. The used fluids also gave similar responses although TMS-9 was somewhat more potent. In the epidemiologic studies, workers in the plant using T-Kool had higher response rates. Thus, qualitative differences between the used fluids cannot explain the epidemiologic findings.

## DISCUSSANT'S COMMENTS and OPEN DISCUSSION

**Dr. FRANKLIN MIRER, UAW:** I have been given the honor of introducing the Discussants, which I guess I'll do in order as they step to the podium. The first Discussant is an old friend. He was doing occupational health on the 14th floor at the Harvard School of Public Health when I was trapped cutting the heads off rats in the basement and that convinced me to go into occupational health. Dr. Larry Fine, Director, Division of Surveillance, NIOSH.

**Dr. LAWRENCE FINE, NIOSH:** Thank you, Frank. My discussion of the last several papers will be very brief since I am not a toxicologist, I am really standing in for Rick Niemeier [of NIOSH], who is a very able and capable toxicologist. As I saw it the first group of papers we heard focused on specific agents and the second group of papers, particularly the latter two, focused on mixtures. I think the total picture we got this morning is a little bit clearer concerning the central message of the respiratory toxicology papers compared to some of the other toxicology papers. The picture I drew from the respiratory toxicology papers, which I think is important and which I think will be echoed this afternoon, is that there is probably not one single mechanism or one single agent that will explain the respiratory effects that the epidemiological studies have identified. Both the toxicology and the epidemiological papers suggest there are several mechanisms and likely more than one agent.

And the second point I'd like to make about that, is that in comparing the two sets of papers, I think we may have more luck getting agreement between the respiratory toxicology studies and the human epidemiological respiratory studies because in both cases we may be looking at acute or subacute effects, rather than effects that have a very long latency period, as do the occupational cancer epidemiological studies. The cancer studies

are focused primarily on exposures that occurred many years ago because of the long induction or latency time associated with development of human cancer.

So I think we should expect to see greater clarity in the area of respiratory effects compared to cancer. I think that clarity will emerge when we compare the messages we got in the last couple of talks with what we will hear this afternoon. But I will let all of you judge that at the end of the day. Thank you all very much.

**DR. FRANKLIN MIRER, UAW:** Our next Discussant is Dr. William Lucke, Manager of Regulatory Affairs, Cincinnati Milacron, representing ILMA. Dr. Lucke tells me he has ventured into enemy territory here, having been educated at Ohio State, but nevertheless, he's willing to step into the breach here. [A reference to the great University of Michigan / Ohio State football rivalry.]

**Dr. WILLIAM LUCKE, Cincinnati Milacron:** A typical union distortion. My undergraduate work was at the University of Nebraska and they still are number one. [A reference to the national ranking of Nebraska's football team.]

John Howell mentioned yesterday that there is a market out there of about 80 million gallons of metalworking fluids or metal removal fluids. That is really too small to suit the people in the industry. It's also not large enough to support very much in the way of product development by the chemical industries. What we do to get around that is to use chemicals that are available for other purposes and that are already being made in quantities so that we can realize somebody else's quantities of scale or economies of scale.

By and large, those chemicals are the same

ones that are used in personal care products. They are available at a reasonable cost and in a reasonable quantity and there's a fair amount of human health data out there on them so that we can go to bed at night knowing that our raw materials are used in baby shampoo and toothpaste, shaving cream, laundry detergents and that sort of thing and know that we're not going to be looking at Mike Wallace the next morning because if there is a problem, he'll go to Proctor & Gamble, not to Cincinnati Milacron.

Nonetheless, there are times when problems come up and I think Dr. Bucher gave us some examples this morning and I think it's informative to see how industry has responded to these things. In the case of the chlorinated paraffins, I'm not aware of anybody putting a product on the market that requires a carcinogen label. Those materials were removed in 1985 prior to the implementation of the Hazard Communication Standard.

We're not entirely driven by regulations, certainly. In the case of diethanolamine, when the NTP study results became available, there was a general movement in the industry to either reduce the amount of diethanolamine in the products to get a bigger safety factor, or to eliminate them completely, where that was possible.

We have the example now this morning of some questionable results with lauric acid DEA condensate and I can't say that that's being used by anybody in the industry, but certainly there is a red flag up on that chemical or similar chemicals and these are things that are going to be looked at in the future. We won't wait for the rest of the animal studies to come up. Something will happen.

The example that really occurs to me, though, is the nitrosamine situation. Twenty years ago nobody was aware of this. The attention of the agencies to the nitrosamines was actually initiated by the industry. It was a rather tortured route because we didn't have TSCA 8e reports in those days. We didn't have a Hazard Communication Standard. There was no formal way to make this known, but still it was made known. And between September of 1976 and November 25th of 1985, the nitrated fluids disappeared from the market.

The equivalent of this would be the changeover from leaded gasoline to unleaded gasoline within the automobile industry. It took a period of time to convert the engines of the cars that were being sold to burn an unleaded gas. It took a longer period of time for the older cars to get off the road and for leaded gasoline to stop being available at the pump. But I think our turnaround was exemplary in that case.

We have a similar situation going today now with the question of respiratory irritation. The UAW has asked for a limit to be lowered to 0.5 milligrams per cubic meter from five milligrams per cubic meter which applies to oil mist, not to metalworking fluids, or the similar five milligrams per cubic meter for respirable aerosols. We support that. We have been advocating it prior to this within our own industry. Our data indicate that we have, what I will call, an AL100, the level at which 100 percent of the exposed population is aggravated. For a metal-working mist, that comes to about three milligrams per cubic meter. If you have an operator exposed to those levels, he's angry, he's upset. If you go into his plant and he sees you monitoring, he will come across the plant and take you to his machine and say "give me some data here."

We're looking at a proposed voluntary lowering of the guideline. We don't know to what level. We think it should be two milligrams per cubic meter or less. We know that the levels in the industry right now are averaging about one milligram per cubic meter. In most cases, I think this is adequate, but as you are hearing this morning, there are two questions here. There is irritation from a chemical source and there is irritation from a biological source. Lowering the limit will help on the chemical irritation, but our experience has been that the irritation that you can associate with endotoxins and other biological agents is not limited to the machine site. Somebody removed from the machine is going to have problems with that sort of thing. So you need changes in ventilation, you need changes in machine placement and other things.

The question of using an animal test to set that limit I think is suspect. If nothing else, we had

the example this morning in Dr. Schaper's slide of isononanoic acid for which she recommended an OEL of seven milligrams per cubic meter. We can tell you that in formulating, if you go over three percent of the concentrate as isononanoic acid, stand by your phone, people are going to call you. This is at, say, a one milligram per cubic meter level of mist. Three percent of that [1 mg] gets you down to 0.03 milligrams per cubic meter of the acid and that is unacceptable. The people will tell you that. They haven't been to college the way the mice have, but that's a practical limit.

Knowing that the animal tests do not predict the known irritants, even though we have consistency in the papers by Schaper and Ball in that they identified the amides, the sulfonates and the tall oil fatty acids as being irritants, we're not sure that we can trust those answers. Thank you.

**Dr. FRANKLIN MIRER, UAW:** Thank you very much. I think that was a very useful comment and hopefully we can work together to move forward.

The next Discussant is Larry Roslinski. Dr. Larry Roslinski is Head of Toxicology for Ford Motor Company. We have been hanging around the glass house together for near 20 years now and I look forward to his comments.

**Dr. LAWRENCE ROSLINSKI, Ford:** Thank's Frank. One of the things we haven't done is differentiate the toxicologists and epidemiologists for the people here not into the basic biological sciences, and I thought that probably the best way to do it would be by example and it's a timely example because everybody in Michigan knows, probably a lot of people outside of Michigan know, that tomorrow is hunting season for rifle, for deer in Michigan.

And it was about a year ago at this time in my example that I'll use, that two epidemiologists and a biostatistician went out to get a deer in Michigan and they tend to hang around together anyway, but they went hunting this time and they came across a clearing and about 50 yards out was a great big buck with a nice rack of antlers, Both

epidemiologists fired at the same time and the first one missed the deer by about two centimeters across the front of the nose and the second epidemiologist missed him by about two centimeters right behind the tail and the biostatistician says, "We got him dead center." Toxicologists would never do that.

I'll probably be a little bit redundant with what I've got to say because we have all heard the same thing and can analyze it the same way, but I think it's good to go back and recap.

As to what we have heard yesterday and part of today, the complex thing called metalworking fluids is changing. And we see things like the nitrites coming out of the fluids in the late seventies, the chloroparaffins probably in the late seventies and early eighties. We have seen better refining of petroleum stocks, starting probably in the fifties, so 40, 45 years ago. We have also seen changes in the makeup of the PNAs and PACs as information came through IARC and the Hazard Communication Standard came in in the early eighties.

I think we can generally agree, although we can't quantify it, that pre-1970 exposures were probably higher than they are now. There were less controls, and there was poorer hygiene and housekeeping, both with the maintenance of the facility and the fluids.

What have we discussed today then? I think one of the things that seems to be constant is that we don't want to use C<sub>12</sub> 60 percent chlorine saturated chloroparaffins, and from what I've heard before, those have been phased out. I think what's exciting is the ability to begin to quantify the irritation. Irritation has been, in the past in the animal models as with humans, a subjective thing. But we're starting to get data that can start to quantify this, at least in the toxicology sense, and I think that's good. We are starting to get some metrics, rather than subjectivity at least in the animal models and later this afternoon we'll hear about the respiratory epidemiology and perhaps get some insights to be able to compare the two.

We're looking not only at the changing gamish of components in metalworking fluids, but also what happens to them, the difference between

the virgin fluids as they are put into the operation and the used fluids when you take them out and either refine, re-refine, or recycle them. So it's a complex area. We're looking at two things. We're looking at the chronic effects and we're looking at what chemicals or what entities in the fluids might cause or might have caused those effects. Are they still there, are they still worthy of control, or is it something that we have seen in the past, but that is no longer in there now. I think the next couple of days will shed some more light on that.

We are seeing irritation with things that would not be unusual to see irritation with. Let's look at some of the analogies between the cosmetics and metalworking fluids. Take hand soap, for example, you're probably dealing with pHs somewhere around nine or nine and a half. If you get soap in your eyes, in an acute sense, or soak in it, in a subchronic sense, you get irritation and inflammation, and you would be seeing the same kinds of things with fluids that are using the same kind of surfactants, the same kind of alkalinity. So it's not that you wouldn't expect these effects, at least in the qualitative sense.

As for the things that I would like to see in the future, perhaps we can shed some light on it in the discussion that will follow. How does the sensory and pulmonary quantification techniques work when the endotoxins are added into the mix? I had some side bar conversations about that already. We have got the chemical ingredients that are going in to the basic fluids, the used fluids themselves and maybe some things that are dissolved in the fluids, pH changes, and now we're including some little dead bodies of bacteria, the endotoxins that complicate this even more. Can we do some quantification in the sense of mist inhalation? I think that's important.

What it really boils down to is -- are we measuring the right stuff? We are talking about regulating oil mist and from some of the quantitative tests we have heard today, oil is probably the least of our concerns. I think it's a good question that has got to be addressed further. Even more importantly, are we controlling the right things? What if we are measuring and controlling something, and it's not the right

ingredient, or the material as a whole is totally different? Hopefully, we will hear some more about that in this afternoon's discussions on the respiratory epidemiology.

And lastly I think what's becoming clear, and it will probably be reinforced in the next day or two, is that multiple mechanisms probably apply. We're not looking at one continuous biological model that can be used, or one chemical model that can be extrapolated, we are going to be looking at multiple things based on what end points we are concerned with, and based on what is a changing background of the chemistry. We'll hear from the sampling people. We will probably even see some differences in what we are sampling today. Hopefully we will be able to project what should be sampled for in the future and most importantly, what should be restrained or restricted in its use in the future. Thank you.

**DR. FRANKLIN MIRER, UAW:** Our final Discussant is a colleague of 20 years and a dear friend, Peg Seminario, Director of the Occupational Safety and Health Department of the AFL-CIO. It took all kinds of wheedling and begging to get her to leave the scene of the crime in Washington, the crime that's going on today [in reference to the Fed. Govt. budget conflict]. Peg started her career as an Industrial Hygienist and then came to the AFL-CIO from the Harvard School of Public Health about almost 20 years ago now.

**Ms. MARGARET SEMINARIO, AFL-CIO:** Thank's very much, Frank. Actually it really doesn't take very much to get me out of Washington. It's not a particularly fun place to be these days.

Just a couple of observations which again are similar to I think what all of us have seen with these various presentations this morning. Number one, as folks have said, that this is a very complex area. Let me say I am not a toxicologist. I have not really dove into the whole metalworking fluids arena in a detailed way prior to this Symposium. But in reading the papers prior to coming here and

looking at all of this, it is indeed an area that is perhaps amongst the most complex to deal with as an occupational health problem, particularly in the context of the regulatory environment in which I work.

In looking at what was presented this morning, I would agree with what has been said on the issues of irritation, and looking at what has come from the toxicity studies, there is probably more agreement and consistency there with respect to the animal studies and what will be presented this afternoon with respect to the human epidemiology.

I was also quite struck that the effects we see are basically across all the different metalworking fluids, and while there may be some differentiation with respect to effect, it's not as though there is one culprit or one particular fluid jumping out as being the particular problem. It's the whole range of fluids which are presenting these particular problems with respect to irritation. Then as these fluids are used and changed and contaminated, the toxicity obviously becomes more problematic.

With respect to carcinogenicity, there is again less information there coming out of the animal data which perhaps in some ways is not as surprising, given that there does not appear, at least from what was presented today, to have been any studies conducted yet to date which actually expose the animals to fluids that are in use as opposed to the chemical components of the fluids.

So we have very little information coming from the animal studies right now with respect to the carcinogenicity and we have a lot more coming from the human epidemiology.

So what do the papers that were presented to us this morning tell us? I think what they tell us as we look at this whole area, we have to look at all of the various fluids that are in use. We have to not only look at the components of them, we have to look at the actual fluids themselves and we have to look at the conditions of use. I think it clearly tells us that the current exposure limit that exists that regulate these substances indeed is inadequate.

Dr. Schaper made some recommendations based on her findings that perhaps we should be

looking at levels in the range of two milligrams to ten milligrams. But then again, that was based upon her experiments using the fluids in their neat form and if you look at the other work that was presented, it would suggest that we need to add a safety factor to those numbers and those recommendations based upon the increased toxicity as these chemicals are used in the workplace.

I think what it also tells us is that just as the issue is scientifically complex, that as we move into issues of control, it is also complex. We have got the chemical manufacturers who are responsible for the components that are going into these fluids, we have the formulators that are putting them together and then we have the end users. And in each of these areas, there are things that can be done and should be done with respect to the reduction in toxicity and the reduction of risk to exposed workers.

Again, I come from the Washington environment, do a lot of work on regulation, and I see with this particular issue moving forward and issues in the context of both political and legal wranglings we get into at OSHA, that we could proceed in a way that would actually bring about a much better situation with respect to workers as far as better warnings, more information, some improvements in formulation and probably most importantly in the use of these chemicals, much better controls with respect to their use in the workplace.

And I would hope that all who are involved with this issue could come out of this particular Symposium moving forward in some cooperative effort and not get into what we have seen before where the chemical manufacturers say it isn't our fault, it's the users, now if they only controlled that stuff, there wouldn't be a problem. Or the formulator saying, "well, it's not our fault, we're only buying from the chemical manufacturers and they're not telling us what the problems are." Where the users basically say, "well, they're giving us chemicals that are problematic."

So I think that clearly, this is a complex area, and a lot of different people are involved in it. But I think that this Symposium is going to be

quite useful in at least trying to get all the information presented, and out of it, hopefully, will come some agreements as how to move forward and deal with what we are all here to do ultimately, and that is to reduce exposures and reduce the risk of workers exposed to metalworking fluids. Thanks very much.

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**Dr. JOHN HOWELL, Castrol:** We're now going to begin the open discussion portion of this morning's session and as we did yesterday afternoon, we would ask those that would like to ask questions to please come forward to one of the three microphones which are in each of the aisles in the auditorium and please before you begin your question, state your name, your affiliation and then if you have a particular person in mind to whom you would like to address that question, then please ask that question of that person directly. We'll begin over in this side of the room.

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**Dr. Gerhard Raabe:** Gerry Raabe, Mobil Oil Corporation. I was very intrigued by what I thought was a fairly elegant analysis by Dr. Schaper and although my question relates to her work, I'm not sure whether she will be able to answer it, so whoever feels most qualified, please.

In the laboratory I presume all those aerosol sizes were standardized to essentially the same size. In other words, you were comparing one micron size particles of all different constituents when you were doing your additivity modeling.

My question is: In the real world in use, do these constituents sort out in the aerosols based on their volume percents, and are the particulate sizes comparable. In other words, how real world does that laboratory scenario translate to in the machine shop.

**Dr. MICHELLE SCHAPER:** In answer to your question, in terms of the first part, yes, there was uniformity in the way that things were done. We are consistently using the same

generation system and we are using the same impactor to do the sizing, so in answer to the first part, they are done in the same manner, around one micron. That's what Dr. Thorne also said.

In answer to the second part, and I don't know all the components, and I'm not aware that there's much component data out there. But we believe that in terms of the fluids themselves, there have been some studies, I think some of which will be discussed this afternoon, to indicate that with regard to workplace exposures, aerosol sizes are one micron or less. So I don't think it's unrealistic.

**Dr. Raabe:** That's very good because I was very intrigued to see how close you got on the additivity modeling with what is now a very old ACGIH model.

**Dr. SCHAPER:** Yes. I think it worked very well here.

**Dr. WILLIAM LUCKE:** I would like to add a comment to that, if I could. In the poster session tomorrow night there's a paper by some people of Rohm and Haas showing some definite migration selectively of components into the particulate phase, in a situation where you are not pushing the bulk fluid through the nebulizer, so I think that is another factor that is coming out now.

**Dr. JOHN HOWELL:** Very good.

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**Dr. John Phillips:** I'm John Phillips from Dow Chemical and this is just for one of the toxicologists. It's obvious to me that exposure controls is the answer to the question in this situation, but I'm wondering more about the toxicologic characterization of the aerosol in the workplace itself similar to the question that was just asked.

We have seen that the metalworking fluids are a variety of composition and this is further complicated by changes in use, volatilization of the components, as well as physical chemical decomposition and biochemical decomposition.

So the workers become exposed to this gamish of chemicals that's aerosolized and they inhale it or get it on their skins.

So, I'm wondering whether there would be some value in conducting a study right in a plant facility with animals to try and characterize this toxicity. Taking the animals out of the laboratory, getting some inhalation chambers, drawing samples in and comparing that to controls. I know this is unconventional, and we have some knowledge of the components, but the actual gamish that the workers are exposed to may help the complex issue, if we could characterize that.

**Dr. JOHN HOWELL:** Would anyone care to take that one?

**Dr. PETER THORNE:** Well, I guess I'll be the sacrificial lamb. It has been done, I understand occasionally, and part of the reason for the complication is that we're required to do these studies with animals that are treated much better than we treat ourselves in terms of ideal diet, and ideal housing conditions.

Standards of good practice indicate that we have to monitor them serologically so that they are shown to be free of certain diseases, so it's hard to do a study that adheres to those sorts of quality assurance guidelines while having these animals in a plant. So if you can free yourself from that side of it, the next point is one of considering the dose response notion of this.

We normally conduct the studies at higher levels of exposure and then use them to extrapolate to lower levels, and this has to do with the dosimetry of the animals in terms of their lung capacity and how often they breath per minute and this then, if you look at lung burdens, micrograms of compound X deposited per day of exposure, this requires us to operate these at higher levels of exposure than you have in the plant, so the model might not be analogous.

So those are, I guess, what comes to my mind. Anybody else want to comment?

**Dr. DONALD MILTON:** Yes. That last issue was the one I wanted to comment about, in

that some colleagues in the Harvard School of Public Health have recently developed an aerosol concentrator for this very kind of experiment and they're mostly looking at ambient air pollution and taking it to, for example, Los Angeles, and exposing animals to high concentrations of air from polluted environments, but this same approach could be used in an industrial environment and it's actually designed for that purpose. And that may be worth pursuing.

**Dr. JOHN HOWELL:** Thank you very much, gentlemen.

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**Ms. Cathy Walker:** I'm Cathy Walker. I'm with the Canadian Auto Workers in Toronto and I've got a comment and a question and they both concern OELs.

Within Canada, this is regulated provincially predominantly and in every jurisdiction the present law is not stringent enough to protect workers' health. We have proposed an OEL in every jurisdiction where OELs are under review of 0.2 milligrams per cubic meter and the reason is simply that that is the number at which, in Susan Kennedy's study of cross-shift pulmonary function changes 0.2 was the level at which these changes were noted. So far, unfortunately, our lobbying efforts have been totally unsuccessful.

Fortunately within workplaces, we have been able to make very significant improvements in a number of workplaces in getting the exposures down. It's a rare workplace where consistently we're below 0.2, but in a lot of them we have really made very significant progress.

My question, however, is to Dr. Schaper and that I was rather surprised to see the calculation of the OELs being rather dramatically higher than I might have thought they would be in your research and I think that one of the puzzles I have is with the number of 60 that was used to do the calculation. Why not five, why not 1,000? Where does 60 come from because that certainly is generating a much higher OEL than I would have suspected.

**Dr. MICHELLE SCHAPER:** The facts, as I mentioned, we started from discussion with 33. That comes from an abundance of data with sensory irritants. I said then that a larger safety factor is necessary for pulmonary irritants and there is a limited database for that. The work has come from some studies on isocyanates which 60 was proposed to protect workers from the pulmonary irritating effects of those isocyanates.

So that factor, there is limited information for that. It's one of the areas that I have been trying to look at in terms of developing an animal model for pure pulmonary irritants and coming up with the appropriate factor. Indeed it may need to be larger, such as 100.

**Ms. ANN BALL:** I would like to make a brief comment about that. As I noted in my talk about the tolyltriazol being in our formulations at a 0.2 to 0.4 percentage rate in the concentrate if you recall, and I don't remember exactly what the OEL was, it was quite high for tolyltriazol according to that and what we know from field exposure, those OELs, and I think it was ten or above, I don't remember exactly; that would be a very high exposure level and you would have extreme complaints at that, so I don't really think that that's a high enough safety factor in that matter.

**Dr. JOHN HOWELL:** Okay, very good. Thank you very much.

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**Dr. Christopher Skisak:** Chris Skisak, Penzoil Company. If I've learned nothing this morning, it appears that as these new exposure limits are recommended, that we have to be very careful not to generically classify this as a PEL for oil mist because it appears that at least for the oil itself, that's the least of the worries, at least, from a respiratory irritant point of view.

But my question, and it may have been partially answered, I wondered about that 60-fold safety factor on the models. I thought the models were very good, but I wonder why we chose those safety factors. Unlike carcinogenesis, when there

are more uncertainties when you're using a conservative model, obligatory nose breathers, increase of respiration rate, things that would be more conservative than less conservative, why those safety factors in the 60-fold for isocyanates, those are considered allergic hypersensitizers. We're not talking about that this morning with the metalworking fluids at least, based on the data that I have seen presented this morning.

**Dr. MICHELLE SCHAPER:** The factor of 60 from those isocyanates though was not set or was not prepared on the basis of sensitization. Those were strictly irritation type studies.

Now, the issue of 60 or 33, I think the first thing that we have to discuss as far as the safety factor for the sensory irritation was the 33. That number is not pulled out of the air. It's not for one chemical or two chemicals.

I published a very large database in 1993 in the American Industrial Hygiene Association Journal that looked at the sensory and pulmonary irritating properties of about 300 materials, some pure chemicals. I guess the majority (250, I think) were pure materials. Fifty were mixtures. And looking then for those which an RD<sub>50</sub> could be established on the basis of sensory irritation to look at it with respect to the ACGIH TLV also on the basis of sensory irritation. When you put them against each other, that's where the 0.03 comes from. It is the correlation between the two. So in mice, if you take the RD<sub>50</sub> and multiply by 0.03 or divided by 33, if you would like to say that, and correlate that against the TLV, an excellent correlation is established, and that is out there for at least 89 chemicals for which all the data is in.

So it's not pulled out of the air and so some of these other studies which have larger safety factors may be for other reasons, but this number has a concrete basis.

**Dr. Skisak:** Although the ACGIH levels which were set based on irritation, those levels may not have been as concrete. And by the way, I'm not saying that you don't lower your PEL here. I'm just talking about the scientific validity of your methodology more than anything else.

**Dr. SCHAPER:** Well, I'm trying to justify where the 33 is not just a made up number, okay?

**Dr. Skisak:** Okay. At least I know where it comes from. Thank you.

**Dr. JOHN HOWELL:** Thank you very much, Michelle.

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**Mr. Ike Tripp:** Thank you. Ike Tripp of Etna Products. This is a question for Dr. Schaper. She may as well not give up the microphone.

In your talk you presented sensory and pulmonary data for neet aerosolized metalworking fluids. That is these fluids were not cut back with water. I was wondering if you could summarize any data you may have generated on those same metalworking fluids when they are cut back in water, which is, of course, the manner in which they are utilized within the facilities and really, I think that is the primary question that a number of our attendees from the plants have come to hear.

**Dr. MICHELLE SCHAPER:** Okay. That's an easy one. For the metalworking fluids, they were given to us as neet fluids, which meant that they were undiluted and we used them as such and the concentration response curves that you saw here were on those fluids.

Now, I have done work with diluting them and that's no problem. The same fluids may be tested in that manner, if you like. And also, I was given some, I didn't have time for everything today either, but there were some in-use fluids that were given to us and of course were very much diluted because they had been collected in the workplace.

The first thing that happens is when you dilute it, when you try to aerosolize it and to get concentrations to get that whole curve, you basically start to cut off the upper end because you're not putting up as much of the other materials.

Remember, when we do the experiment and we sample on the filters, we are not collecting

the water portion. We are sampling for the solids and nonvaporized components. So when you have more water there, you are reducing, in essence, that amount that you catch on the filters so in order to do the experiment, you're going to have to push more of that fluid through the generator to get enough material up. But the curves are identical, whether you do them with the neet or with the diluted. It's just a matter of trying to push that upper end. Does that make sense to you?

**Mr. Tripp:** Thank you.

**Dr. SCHAPER:** I want to add one other thing, too, is that in terms of our exposure system, people always ask why don't you measure water or where's the water going. We use, remember I said 20 liters a minute for ventilation for that, so if you put up water with that generation system, you change it into water vapor, okay, so there's no water mist left and you can do an experiment with water alone under those conditions and you sample nothing on the filters.

**Dr. JOHN HOWELL:** Thank you, Michelle. Okay.

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**Dr. Dennis O'Conner:** Dennis O'Conner with Exxon. I just want to offer a comment in response to Margaret Seminario's call for information in the sense on fully formulated products and their carcinogenic activity.

We have looked at our base oils and commercially produced products that are derived or produced using those base oils and we've also looked in parallel at used, those formulated products following use, and we have evaluated them and animal bioassays and essentially the data we have would suggest that if you are using noncarcinogenic base oils and noncarcinogenic components, that you are continuing to see no evidence, no increase in carcinogenic potential in the used fluids, if you evaluate them as well in parallel bioassays.

So there are data that do support that. They

are not voluminous, but that has been looked at and that's what it supports.

**Dr. JOHN HOWELL:** Thank you very much, Dennis.

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**Dr. Franklin Mirer:** Frank Mirer from the UAW, taking another perspective on things from the floor.

My question is going to be for Dr. Bucher and Dr. Stott and I wanted to start with the observation for those of us in the business. The target organ toxicity disclosed in their presentations does have to be disclosed on the Material Safety Data Sheet and on the label and that would include all the single species carcinogenicity experiments, including the heavy chlorinated paraffins and triethanolamine.

I would like to compliment Dr. Bucher on the NTP studies and on his effort to come here and present them. Having said that, my question is this: The way I see the triethanolamine study, the dose was limited by how much the animals could tolerate on their skin and as a result, we may not have fully evaluated the potential of this material by inhalation as people are exposed in the industrial environment.

Secondly, we end up with several equivocal studies which are of concern. You certainly raise concern for the potential of carcinogenic effect if a higher dose was applied and third, we had criticism of the study because of the concurrent infection, although I would note that the female mice had hepatitis in the liver, tumors -- I'm sorry, the male mice had hepatitis in the liver tumors and the female mice did not, and did have the liver tumors, so I'm not clear how badly off we are with this study.

So this leads up to the question of whether the alkanolamines panel and NTP either alone or together can get us predictive MTD inhalation study of the effects of triethanolamine, which is clearly on the block here, given the effect seen in people.

**Dr. JOHN BUCHER:** Frank always asks interesting questions. Being in the middle of re-evaluating or continuing to evaluate the current study that we have done on triethanolamines, I mean, it is very difficult for me to sit here and try to predict what we'll do next.

With regard to an inhalation study, we did do the 14-day studies by inhalation of the triethanolamine. It was our understanding and our opinion at the time that we could achieve high relevant doses that would simulate what people were achieving through the use of cosmetics by a dermal route of administration. It's possible that something else could be learned in an inhalation, carcinogenicity study with triethanolamine, but this is a very vexing compound. You saw that there were five bioassays that were done on this compound so far.

There's been a very poor reproducibility in those bioassays in terms of target organ toxicity. You just don't seem to see the same thing showing up. They all have flaws here and there. The Japanese mouse study was only run for 82 weeks, which is not sufficiently long to really be a complete negative.

The Maekawa study in rats was probably a pretty good negative and that's one percent in the water, which is a very high dose. Triethanolamine is almost not metabolized and it seems very difficult to me to understand why with a chemical that seems to get into the body by so many ports of entry, why we need to systematically eliminate each one of those from consideration for carcinogenicity, but these are all good questions and I'm sure we'll see this as a formal nominant. We already have seen this, in fact, as a formal nomination of our program, so we'll be getting back to you with a response, Frank.

**Dr. WILLIAM STOTT:** I guess I would second most to what Dr. Bucher said, other than a few misconceptions in that statement. Number one, inhalation does not represent a major exposure route for the alkanolamines. It is very minor relative to dermal. It's a misconception that I held at one time too when I first saw the data. So dermal far outweighs inhalation as a route of

exposure.

Number two, as Dr. Bucher alluded to as well, that finding in female mouse is not a definite yet and I don't think you can rule out the potential for hepatitis in those animals, so it's not quite as clear as it might seem on just the number comparison.

**Dr. Mirer:** The issue regarding route, not that you have to test everything by every route, but that the skin lesions were the limiting factor in the dose that could be applied to the skin, and that by inhalation or for that matter by drinking water, the animals might have tolerated a larger dose, which might have got the equivocal studies up from equivocal to a better result.

The other comment with regard to the alkanolamines group is that we must have spent, what, two million dollars of taxpayers money testing your chemical that millions of people are exposed to every day in their shampoo and also quite a few hundred thousand in the workplace, so having trashed or attempted to trash the NTP bioassay, what's the industry going to do to replace it with an equivalent chronic test using mortality adjusted statistics, using a group reviewed MTD, using peer reviewed levels of evidence at the end. What's the industry going to do to correct this gap in what is now, in my view, a pretty clear one species carcinogen.

**Dr. STOTT:** It's not in everybody's view, okay? Also --

**Dr. Mirer:** I'm aware of that.

**Dr. STOTT:** When you're looking at that, as Dr. Bucher indicated, the weight of the data there doesn't seem to warrant beating on this compound much more. Speaking of beating up on an assay, that's just good scientific review of the data. That's not trashing anything. We don't "trash" a study. We review it, so professionally I've got to answer that one, okay? But there's a lot of data out there that says you are pointing at the wrong place, if there's anything to point at.

**Dr. JOHN HOWELL:** Okay. Thank you very much, gentlemen.

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**Dr. David Steup:** Dave Steup, Shell Oil. I have a question for Dr. Milton. I was interested in your results that seemed to show a kind of multiple mechanism type of effect, in that you showed some effects which were pretty clearly related to the endotoxin and could be enhanced or decreased, and kind of a base line of possibly nonspecific or another mechanism or effect that really wasn't accounted for by it.

It's well known that hydrocarbons introduced into the lung can cause pulmonary injury simply by interfering with pulmonary surfactant and mechanisms of this type, and this is usually an effect with respirable type hydrocarbon materials of low viscosity, but in this case, you are introducing an emulsion containing a standardized amount of oil directly into the lung.

Have you considered or done any controls to address the possibility that this might be just a rather nonspecific effect which is to some extent an artifact of your technique by, say running a control of the right highly refined low reactivity, something like a food grade white oil or something like that.

**Dr. DONALD MILTON:** Those experiments have been done not as part of the series that I presented here and in general, such as corn oil has been used and is a much lower toxicity in this assay than what we found with the metalworking fluids.

**Dr. Steup:** I would argue, though, that corn oil might not be the appropriate control. It's certainly a more biologically reactive material and would not necessarily give you the same kind of effect that you might see with a mineral hydrocarbon.

**Dr. MILTON:** I believe we have also done mineral oil as well and seen relatively low

toxicity in that case.

**Dr. JOHN HOWELL:** Okay. Thank you very much.

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**Mr. Robert Fensterheim:** Bob Fensterheim with RegNet Environmental Services, but I'm here today with my hat on as the Executive Director of the Chlorinated Paraffins Industry Association.

I just want to offer a clarification on some statements that were made. Several times there were some statements which suggested that chlorinated paraffins are no longer used. I think most of the people in this room would recognize that they are still widely used in metalworking fluids. What's important in talking about chlorinated paraffins, because they do represent a very broad category of chemicals with varying physical chemical and biological properties as evidenced by Dr. Bucher's presentation, is that it is true that there has been a dramatic drop in the use of the 12 carbon, 60 percent chlorinated paraffin which was found to be a suspect carcinogen in the NTP bioassay. In talking about chlorinated paraffins, I would really encourage people to at a minimum distinguish between short, intermediate and long materials. Chlorinated paraffins are comprised of compounds that vary all the way from ten carbon to 30 carbon materials and so as you can imagine, you get very different properties from that and it is the short chain material, the ten to 13 carbon material that's been the focus of attention, and I missed John Howell's presentation earlier, but I think he talked about the [EPA] Toxic Release Inventory and it's really just been the short chain that's been the focus of attention.

**Dr. JOHN HOWELL:** Okay, thank you very much.

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**Dr. Richard Kraska:** I'm Rich Kraska, a Toxicologist with the Lubrizol Corporation and I just wanted to add some further discussion about cancer bioassays. Frank [Mirer] mentioned that perhaps we wasted two million dollars on an NTP diethanolamine assay. I don't think we really wasted the money.

There was a recent editorial in Science Magazine that suggested that we have wasted a trillion dollars on studying cancer in animals and I'm not sure they really meant that they wasted the money, but I don't think we really have what anyone would agree would be a trillion dollars worth of knowledge about the carcinogenicity of chemicals and a lot of that is just based on our imperfect understanding. And I don't really mean to say that and to accuse anyone of wasting money. I run my own tox program at my own company and I don't think I've gotten my company's money's worth just based on bad advice from the regulatory agencies on protocols and the way things should be done.

But certainly we need more carcinogenicity data in animals and I would disagree with Frank. I think we should have it from all routes of exposure because I think the routes of exposure are important, but at this point in time with our knowledge of the science, we're still at a loss in order to know how to study these chemicals.

There's raging controversies about corn oil gavage and caloric intake in these animal model systems. There's also raging debates on whether the animal models have drifted to getting more obese animals and whether that's skewing the data set. A lot of the end points that were discussed today, male rat kidney and mouse liver tumors in toxicological circles, those are very suspect end points for a variety of different reasons.

So I guess I agree with everyone that we need more data. The problem is at this point in the science, we really hesitate to know how to proceed without some more basic research on what are the appropriate animal models and test protocols.

**Dr. JOHN HOWELL:** Thank you very much.

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**Dr. Sridhar Reddy:** Sridhar Reddy from Wayne State University Detroit. My question relates to the availability of animal data on stuff that's going to be presented in the afternoon. I guess you are going to be presenting human data on occupational asthma and I'm wondering if people have been looking into animal models of asthma or hypersensitivity pneumonitis. Basically pulmonary sensitization and metalworking fluids. I'd like the presenters who have looked into the animal models to comment on that. Thank you.

**Dr. PETER THORNE:** I think Michelle and I are both going to comment on that. Certainly there are animal models for that that have been developed and widely used and both of us are among those investigators that do use these models. In terms of hypersensitivity pneumonitis I have studied this in guinea pig and mouse in the context of dairy barns and exposure to thermophillic organisms that are responsible for farmer's lung disease. Those are very well established models, but I haven't applied those to this particular arena. So that's farmer's lung disease hypersensitivity pneumonitis.

Looking at pulmonary hypersensitivity in terms of airway responses, I didn't have the opportunity to talk about that at length here, but did mention that in testing the metalworking fluids and here in-use fluids, I did not find any sort of response in a maximization type approach for pulmonary hypersensitivity. I did also study biocides and there was some equivocal results for some of the biocides at high levels of exposure and so I know that there are some studies going on currently to look further at the biocides.

The studies that I did there would look at pulmonary evidence based on pulmonary responses. The next step, of course, is to look at it immunologically and we're going to be doing that, but I don't have anything that I could report on the immunology of it at this point.

**Dr. MICHELLE SCHAPER:** We also have looked at what Peter described in the last part of his answer to you, pulmonary sensitization of guinea pigs to aerosols and metalworking fluids. We tried to sensitize guinea pigs I believe to four of these seven different metalworking fluids. We published an abstract, and I believe if you look in your extended abstract [reference #8, M. Schaper's paper - page 93 this Proceedings], it will be around 1993 and it was at the Society of Toxicology meeting where it was presented.

One of the four we did find some evidence of pulmonary sensitization. Now I don't recall exactly which one it was. I don't think it was A, B or my recollection is that it was not A, B or E, but it was one of the other soluble fluids. So the question in our mind is did it contain something that was a pulmonary sensitizer, but only one of the four.

**Dr. Reddy:** Thank you very much.

**Dr. JOHN HOWELL:** Very good. Thank you very much.

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**Mr. Kenneth Cavanaugh:** Ken Cavanaugh from Houghton International. This is addressed to Dr. Thorne or Dr. Milton. You presented some very good information on the respiratory affects of various used metalworking fluids when these metalworking fluids contain a wide variety of various living microbes when they are sampled.

I was wondering what methods are used to preserve these samples when they are taken, in order that there will be no change until you take them to your laboratories and run the inhalation studies with the lab animals.

**Dr. PETER THORNE:** We'll each answer that individually, I guess, and we'll see if the answers are the same. We were doing these studies at the same time we were doing in-plant studies, so we had staff at the lab ready to run the assays and we were in the field collecting the

samples. We collected them and we shipped them back and we did the studies the next day.

In some cases we had people at the plant collect the fluids for us and chill them and ship them overnight and we did the study the next day. So we were concerned as to what was happening with these fluids in terms of the endotoxin concentration and the levels of bacteria.

So we took 18 of the fluid samples and we analyzed them for fungi, bacteria and gram negative bacteria and endotoxin concentration weekly for two months on those 18 and what we found was that there was a decline in the concentration of gram negative bacteria and mesophilic organisms because there was residual biocide present in most cases and these would gradually decline until they reached our lower limit of detection over the course of two months.

In some cases they did not reach the lower limit of detection and some they did, but these were changes that occurred over some time and we found that one day lag in performing the inhalation studies had virtually no effect on the concentration of these organisms. We didn't get significant decline in that time frame.

**Dr. DONALD MILTON:** In our samples, we found that over a period of about four months the endotoxin levels declined in some samples and others remained relatively constant. In the samples that we used here, we were not able to do it in the timely fashion that Peter was able to do it, but we did find that the endotoxin and concentrations were stable over the time period of the study.

**Mr. Cavanaugh:** Thank you.

**Dr. JOHN HOWELL:** Very good. Thank you very much. Are there any more questions from the participants in the auditorium this morning? Okay, very good. Do any of the Discussants have any further comments regarding what you have heard this morning? Sounds like we don't. Very good. What we'll be doing then is adjourning the morning session here. Please be back in your seats for a prompt 1:15 p.m. start.

