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Ms. Diane Manning
NIOSH, Education and Information Division
Mail Stop C34
4676 Columbia Parkway
Cincinnati, OH 45226-1998

April 26, 1996

Dear Ms. Manning:

In response to the letter of Dr. Lawrence Fine and Draft of the Metalworking Fluid Criteria Document (February 1996), I am sending this cover letter and the attached notebook. I have compiled the abstracts and papers published by our laboratory. In the Draft Criteria Document, two of our studies were cited (one published in 1991 and one that was "in press"). We now have four published papers on metalworking fluids; all were peer-reviewed. It is my hope that Section 5.3 will be updated to incorporate the new results. In addition, I have enclosed the page-proofs of another peer-reviewed paper (Boylstein et al., 1996) that will appear in *Archives of Toxicology* in the near future. I would recommend the citation of this paper in the final version of the Metalworking Fluid Criteria Document. If you have any questions, please do not hesitate to contact me. Thank you very much.

Sincerely,

Michelle M. Schaper

Michelle M. Schaper, Ph.D.
Associate Professor of Environmental and Occupational Health

METALWORKING FLUIDS
PUBLISHED PAPERS AND ABSTRACTS

MICHELLE M. SCHAPER

APRIL, 1996

829 ACUTE RESPIRATORY EFFECTS OF AEROSOLIZED MACHINING FLUIDS IN MICE. M Schaper and K Detwiler, University of Pittsburgh, Pittsburgh, PA.

Using a previously developed bioassay, the sensory and pulmonary irritating properties of a group of 10 aerosolized machining fluids were evaluated in mice during single, 3-hr inhalation exposures. The results showed that all 10 were capable of inducing sensory and pulmonary irritation. Respiratory frequency decreased in a concentration-dependent manner with little change in tidal volume. There was little or no change in pulmonary histopathology. Comparison of concentration-response relationships for the 10 fluids revealed that synthetic/semi-synthetic and soluble fluids were more potent irritants than straight oils. Also, "used" fluids were found to be similar in potency to the corresponding "new" fluids. From these relationships, the RD50 value (i.e., concentration inducing a 50% decrease in respiratory frequency) was obtained for each fluid. Using RD50 values, occupational exposure limits were then suggested. This bioassay may be a good first step to evaluate new machining fluids whose formulations may change depending upon current industrial needs. Supported by the United Auto Workers and General Motors National Joint Committee on Health and Safety.

830 EFFECTS OF MAXIMAL INCREMENTAL EXERCISE ON OXYGEN CONSUMPTION IN SHEEP. T G Mundie, A J Januszkiewicz, D B Rayburn, G R Ripple, D G Martin. Dept. of Respir. Res., Walter Reed Army Institute of Research, Wash., D.C. Sponsor: B E Lehnert.

Exercise stress tests are useful in evaluating occupational lung disease from inhaled toxicants. This study assessed the feasibility of using sheep in assessing oxygen consumption (Vo_2) during maximal incremental treadmill exercise. Six sheep were exercised with three different incremental exercise protocols. Significant differences were found between the three protocols for maximum Vo_2, anaerobic threshold (AT) and maximum minute ventilation (V_E). Mean maximum Vo_2 for 7, 13.5 and 16-min exercise protocols were 49.9 ± 5.0, 37.8 ± 6.5 and 42.3 ± 6.0 ml O_2/min/kg (mean $\pm$ s.d.), respectively. Maximum Vo_2, AT and maximum V_E was highest for the most intense protocol. The sheep were then conditioned for an additional 12 weeks and re-tested using the 13.5-min protocol. Conditioning resulted in increased time on the treadmill, maximum Vo_2, AT and maximum V_E. Maximum Vo_2 was 37.8 ± 6.5 and 48.1 ± 9.1 ml O_2/min/kg before and after additional training. These data demonstrate that the intensity of the incremental test and the extent of endurance training are important factors in the ventilatory response of sheep to maximal exercise. The sheep is suitable for future studies to evaluate pneumotoxin-induced performance decrement.

831 A TWO-GENERATION REPRODUCTION STUDY IN RATS WITH ALKYLATE 215. R E Schroeder, Bio/dynamics, Inc., East Millstone, NJ; E C Robinson, Monsanto Company, St. Louis, MO.

This study was conducted to evaluate the effects of Alkylate 215, a C10-C12 linear alkylbenzene, on the reproductive performance of CD® rats through two generations. Test article was administered by gastric intubation at dosage levels of 5, 50 and 500 mg/kg/day (MPK). Each adult generation (30 animals/sex/group) was mated to produce a single litter; F_1 adults were selected from the F_1 litters. F_0 and F_1 animals received a 10-11 week pre-mating treatment period. Adults and weaned pups received a gross postmortem examination. Reproductive tissues, gross lesions and pituitary were taken from each adult animal and were evaluated histopathologically for control and high-dose animals (F_0, F_1). No adverse effects of treatment were seen at the 5 MPK level. At the 50 MPK level, no consistent effect of treatment was evident through both generations. At the 500 MPK dosage level, the following statistically significant changes were seen through both generations; reduced mean weight gain for adult males during the pre-mating period; reduced litter size and pup viability indices at birth; reduced pup survival indices (Days 0-4); and lower mean pup weights (Days 14 and 21). No adverse effect of treatment to the 500 MPK dosage level was seen from gross postmortem evaluation (adults, pups) or histopathological evaluations (adults).

832 MATERNAL AND EMBRYONIC DISPOSITION OF ORAL TRICHLOROETHYLENE (TCE). CE Dallas, S Muralidhara, XM Chen, *EK Stevens, *JE Martin and *TR Irvin. Pharmacology/Toxicology Dept., U of Georgia, Athens, GA and (*)Vet Anatomy Dept., Texas A&M Univ., Coll Sta, TX. Sponsor: A Ray.

We have evaluated the pharmacokinetics of trichloroethylene (TCE) in pregnant Sprague-Dawley rats following oral exposure on day 11 of pregnancy. After a single TCE dose in PEG of 200 mg/kg, animals were sacrificed as a function of time post dosing, and serial samples of brain, liver, kidney, lung, heart, skeletal muscle, adipose tissue and whole embryos were taken. Tissue samples were homogenized in cold saline, extracted in cold isooctane, and analyzed for TCE using a GC-ECD head space technique. Maximum tissue concentrations (Cmax) were achieved within 30 min of dosing for all tissues. TCE elimination in maternal fat had a significantly higher t1/2 than all of the other tissue groups. Maternal fat AUC and Cmax were 4-11 and 5-32 times, respectively, higher than those in other maternal tissues, and two orders of magnitude greater those in embryonic tissues. [Supported by Air Force grant AFOSR 870248 (CED) and EPA Grants CR816008010 (TRI)].

824 SUBCHRONIC INHALATION EXPOSURE TO AN ORGANIC RED DYE MIXTURE: EFFECTS ON RAT LUNG FUNCTION. D W Winsett, J S Tepper, R H Jaskot, and D L Costa*. NSI-ES, RTP, NC and *EPA, RTP, NC.

Organic dyes are used by the military in colored-smoke marking grenades. The potential inhalation exposure of munitions workers to such dyes, particularly as mixtures, necessitates the assessment of their pulmonary toxicity. A red dye mixture (RDM) consisting of two red dyes, 1,4-diamino-2-methoxy-anthraquinone (Disperse Red 11) and 1-(2-methoxyphenylazo)-2-naphthol (Sudan Red), at a ratio of 9.4:90.6 was delivered as a dry aerosol (MMAD 2.4 μm, σg=2.3) to male F-344 rats (SPF; 90d at the start). Groups of 10 rats were evaluated for lung dysfunction immediately after 4 or 13 wk of exposure (6h/d; 5d/wk at 0, 30, 100, or 300 mg/m^3) or after a period (2 or 4 wk, respectively) in filtered air. Compliance (C_{rs}), peak flow during forced expiration (PKFLOW), and body weight (BW) decreased, while end-expiratory volume increased, after 4 wk of exposure. These effects did not resolve after 2 wk in filtered air. After 13 wk RDM, significant effects were found for C_{rs}↓, PKFLOW↓, N_2 washout↑, total lung capacity↓, vital capacity↓, and BW↓; most of these effects also did not resolve. These data suggest the development of a subtle but significant lung restriction disorder. The lung dysfunction will be correlated with tissue structure/composition. The 300 mg/m^3 RDM exposure group showed the most marked effects, although similar trends were seen at the lower concentrations. (This abstract does not necessarily reflect Army or EPA policy)

825 TOXIC INTERACTIONS OF BINARY MIXTURES OF NO_2 & CO, NO_2 & HCN, NO_2 & REDUCED O_2, AND NO_2 & CO_2. B C Levin, M Paabo, and M Navarro. National Institute of Standards and Technology (NIST), Gaithersburg, MD.

A model to predict the toxic interactions of the major gases produced in fires is being developed at NIST and includes six gases - CO, CO_2, HCN, reduced O_2, HCl, and HBr. The current objective is to add NO_2 to the model. The toxicity of individual gases, CO, CO_2, reduced O_2, HCN, and NO_2, in air as well as various two, three and four gas combinations have been examined in Fischer 344 male rats exposed for 30 min and observed for 14 days. LC_{50}'s for HCl and HBr were obtained from the literature. Deaths from NO_2 in air occurred only in the post-exposure (PE) period and its LC_{50} is 200 ppm (PE). Carbon dioxide has synergistic toxicological effects when combined with any of the other gases tested at NIST. The LC_{50} for NO_2 in the presence of 5% CO_2 is 90 ppm (PE). CO produces only within-exposure deaths (WE) and its 30 min LC_{50} is 6600 ppm. In the presence of 200 ppm of NO_2, the WE toxicity of CO increased. At the new LC_{50} of CO, the PE toxicity of NO_2 also increased. Deaths from HCN occur primarily during or within 24 hours following exposure and its LC_{50}'s are 200 ppm (WE) and 150 ppm (PE). An antagonistic effect is observed with NO_2 and HCN; in the presence of 200 ppm of NO_2, 2.3 to 2.5 times the HCN LC_{50} is needed to produce one death within-exposure. Deaths from reduced O_2 occur primarily within exposure and its 30 min LC_{50} is 5.4%. In the presence of 200 ppm of NO_2, the WE LC_{50} of O_2 and its toxicity increased. At this new LC_{50} of O_2, the PE toxicity of NO_2 increased. Thus, NO_2, a PE toxic gas, increases the toxicity of the WE toxic gases (except HCN) and vice-versa.

826 FURTHER EVALUATION OF PULMONARY EFFECTS OF AEROSOLIZED MACHINING FLUIDS IN MICE AND GUINEA PIGS. K Detwiler and M Schaper. University of Pittsburgh, Graduate School of Public Health, Pittsburgh, PA.

The sensory and pulmonary irritating properties of 10 aerosolized machining fluids were recently evaluated in mice and for each fluid, the concentration capable of evoking a 50% decrease in respiratory rate (RD50) was obtained. Four of these fluids were studied here. Groups of mice were exposed to each fluid at its RD50 on Days 1, 2, 3, 4, 5 and 14 for 3 hours/day. The same relative decrease in respiratory rate was evoked each day; thus, there was no evidence of a cumulative effect. Groups of guinea pigs were also exposed to each fluid at its RD50 on Days 1, 2, 3, 4 and 5 for 30 minutes/day. Similar exposures were conducted on Days 19, 33, 47, 61, and 75. No pulmonary effects were observed on Days 1-5 or on Day 19. However, on Days 33-75, bronchoconstriction occurred in 2/4 animals that were exposed to a semi-synthetic fluid. No response occurred in the other exposed animals. These data suggest that the semi-synthetic fluid may contain a sensitizing ingredient. This protocol may be useful for screening other machining fluids for sensitization potential. Supported by UAW-GM.

827 SUBCHRONIC AND DEVELOPMENTAL TOXICITY OF DERMALLY APPLIED REFINERY WASTE WATER SLUDGES IN RATS. M H Feuston, S L Kerstetter and C E Hamilton. Mobil Oil Corp., Princeton, NJ.

Two refinery waste water sludges, API Separator Bottom Sludge (API Sludge) and Dissolved Air Flotation (DAF) Float, were evaluated for subchronic (SC) and developmental toxicity. In the API Sludge studies, the material was applied to the clipped backs of rats at dose levels of 0, 500 and 2000 mg/kg; dose sites were occluded. Pregnant rats were exposed to the sludge on gestation days (GD) 0-20; in the SC study, male rats were exposed for 90 days, 5 days/wk. In the DAF Float studies, the test material was applied to non-occluded sites at dose levels of 0, 60, 250, and 1000 mg/kg to male and female rats for 90 days, 5 days/wk. Pregnant rats were exposed to DAF Float at 0, 125, and 500 mg/kg on GD 0-20 or to 1000 mg/kg on GD 0-15. In the SC studies, API Sludge did not produce overt signs of toxicity; DAF Float produced skin irritation, decreased body wt, aberrant hematology, altered organ wt and reduced sperm count. Both sludges produced maternal and developmental toxicity. Adverse fetal effects included an increase in intrauterine death, decreased body wt, and reduced skeletal ossification. The systemic and developmental effects may be related to levels of polynuclear aromatic compounds derived from petroleum. Refinery streams containing polynuclear aromatic hydrocarbons (PAH) and PAH containing nitrogen are known to cause SC and developmental toxicity.

1199 **A 2-WEEK REPEATED VAPOR INHALATION TOXICITY STUDY OF HYDROGEN CHLORIDE AND SELECTED CHLOROSILANES IN CD RATS.** W H Siddiqui G B Kolesar M G Evans R G Meeks and R W Mast. Dow Corning Corporation, Midland, Michigan.

Chlorosilanes are used as intermediates for the synthesis of a large number of commercially important silicones. We previously reported (Toxicologist, 7, 768, 1987) the acute inhalation toxicity data of hydrogen chloride gas (HCl) and selected chlorosilanes in rats. The present study was conducted to further characterize the differences in toxicity between HCl and chlorosilanes following repeated inhalation exposures. The exposure concentrations for chlorosilanes were based on HCl equivalents. The chlorosilanes evaluated were methyltrichlorosilane ($MeSiCl_3$), dimethyldichlorosilane (Me_2SiCl_2), trimethylchlorosilane (Me_3SiCl), and trichlorosilane (Cl_3SiH). Groups of 5 male and 5 female rats were exposed in a nose only chamber for 6 hours per day 5 days/week for 14 days to target concentrations of 30, 15, 10, 10, and 30 ppm of Me_3SiCl, Me_2SiCl_2, $MeSiCl_3$, $HSiCl_3$ and HCl respectively.

No mortality or adverse clinical signs were observed in any groups. Necropsy findings were unremarkable. No statistically significant differences were noted in body weight gains or in absolute or relative organ weights. Histopathologic evaluations did not reveal any significant findings in animals exposed to chlorosilanes or to HCl except mineralization in kidneys in female rats exposed to $HSiCl_3$. Whether or not this lesion is related to the administration of $HSiCl_3$ is under investigation.

1200 LIGHT AND ELECTRON MICROSCOPIC CHANGES DURING THE COURSE OF DEVELOPMENT OF PHOSGENE-INDUCED LUNG INJURY. RJ Sebring, DC Archuleta, A Deshpande, and BE Lehnert. Los Alamos National Laboratory, Los Alamos, NM.

The inhalation of phosgene (P) can result in severe pulmonary edema and death. In order to obtain information about the anatomical sites where P exerts its effects in the lung, we exposed groups of Fischer-344 rats for 10 min to 9 or 32 ppm P and then sacrificed the animals for light microscopic and ultrastructural/electron microscopic analyses of their lungs at times ranging from immediately to 48 hrs after exposure. Air exposed rats served as controls. With 9 ppm P, no ultrastructural damage was apparent as of 6 hrs after exposure. From 6 to 48 hrs, damage was confined to the first generations of alveolar ducts. Such injury was characterized by destruction of type I epithelial cells with the subsequent release of septal cells, erythrocytes, and fluid into the alveolar spaces. Abnormal alveolar fluid constituents contained considerable amounts of myelin figures, as well as tubular myelin and fibrin. Very little interstitial edema was noted and no damage to alveolar macrophages or airway epithelial cells was observed, even in the terminal bronchioles. At 48 hrs, tissue repair characterized by type II hyperplasia and significant parenchymal thickening in the damaged regions was apparent. With the 32 ppm of P, obvious injury occurred as early as 1 hr after exposure. Like with 9 ppm P, alveolar damage was generally restricted to the first few alveoli beyond the terminal bronchiole. Unlike with the 9 ppm concentration, severe airway epithelial damage was also observed in the more peripheral regions of the terminal bronchioles. This work was funded by the U.S. DoD and conducted under the auspices of the U.S. D.O.E.

1202 DOUBLING OF INHALED DOSE IN RESTRAINED, OVER UNRESTRAINED, MALE F344 RATS DURING WHOLE BODY EXPOSURES TO CHLOROFORM ($CHCl_3$). Q R Moss, J E Murphy, S J Borghoff. Chemical Industry Institute of Toxicology, Research Triangle Park, NC

Under conditions of whole body inhalation exposures, animals in the past have been exposed either as an unrestrained group in one or more cages , unrestrained but individually housed in multiple cages, or individually restrained in some form of tube placed completely within the chamber. In order to compare different experiments we evaluated the hypothesis that there was no influence of exposure housing configuration on inhaled dose. Male F344 rats were placed into groups of four and exposed for 4 hours in a Leach chamber to 200 ppm of $CHCl_3$. Rats were exposed either singly, as a free roaming group (total of 4), separated in individual cages, or held in cylindrical restraint tubes ("open" nose-only exposure tubes, CH Technology, Westwood, NJ). From 3-14 minutes post exposure blood, liver and kidneys were collected and tissue chloroform concentration measured by vial equilibration and GC headspace analysis. At three minutes post exposure, blood from animals held in cylindrical restraint tubes, inside the whole body inhalation exposure chamber, contained twice the amount of $CHCl_3$ compared to the level in blood from any of the other rats. $CHCl_3$ content in liver and kidneys showed a similar trend. There was no clear statistical difference in the initial tissue levels between the singly exposed, separately caged, and free roaming rats. The unrestrained exposure group size was not large enough to show an enhancement of single animal avoidance behavior such as re-breathing exhaled air. This may not be the case for gases and vapors more reactive than chloroform.

1203 ACUTE RESPIRATORY EFFECTS FROM COMPONENTS OF METALWORKING FLUIDS IN MICE. M Schaper and K A Detwiler-Okabayashi. University of Pittsburgh, Graduate School of Public Health, Pittsburgh, PA.

Previously, the acute respiratory effects of ten aerosolized metalworking fluids were evaluated in mice (Schaper and Detwiler, Fund. Appl. Toxicol. 16, 1991). For each fluid, a concentration-response relationship was developed, and the concentration capable of producing a 50% decrease in respiratory frequency (RD_{50}) was determined. Sample E, one of the ten, was a neat soluble fluid that produced both sensory and pulmonary irritation. Its RD_{50} was 500 mg/m^3. For the present study, we obtained the three major components of Sample E: sulfonic acid (SA), tall oil/fatty acid (TOFA), and paraffinic oil (PO). Each component was aerosolized, and its respiratory ffects were evaluated in mice. RD_{50} values were 100 mg/m^3 for TOFA and SA, and 4,000 mg/m^3 for PO. Based upon potency, type(s) of effects produced, and proportion of these three components in Sample E, the data suggested that respiratory effects of Sample E were due to a combination of TOFA and SA. This approach may be useful for identifying the biologically active ingredient(s) in other metalworking fluids. Supported by United Auto Workers and General Motors.

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IDENTIFICATION OF IRRITANTS PRESENT IN METALWORKING FLUIDS USING AN ANIMAL BIOASSAY. M.M. Schaper, K.A. Detwiler-Okabayashi, and S.P. Krystofiak, University of Pittsburgh, Graduate School of Public Health, Center for Environmental and Occupational Health and Toxicology, 260 Kappa Drive, Pittsburgh, PA 15238.

In a previous study conducted in our laboratory, the acute sensory and pulmonary irritating properties of 10 metalworking fluids were evaluated. Mice were exposed to aerosols of the metalworking fluids for 3 hours, and during these exposures, continuous measurements of animal respiration were made. Using respiratory frequency as the indicator of response, a concentration-response relationship was developed for each of the 10 fluids From these relationships, the concentration capable of producing a 50% decrease in respiratory frequency (RD_{50}) was obtained. On the basis of RD_{50} values, we found significant differences in potency of the 10 fluids as irritants. However, it was not possible to determine the component(s) responsible for the observed irritant effects.

The present study has focused on 1 of the 10 fluids: a semisynthetic fluid ("Neat" Sample B) whose RD_{50} was 150 mg/m^3 *Sample B* has 7 major chemical components that are combined with water: alkanolamide "A" (#1), alkanolamide "B" (#2), boramide (#3), petroleum oil (#4), potassium soap (#5), sodium sulfonate (#6), and triazine (#7) Using the same protocol as described above, mice were exposed to aerosols of each of the 7 components of *Sampie B*, and a concentration-response relationship was developed for each component, except boramide Petroleum oil had an RD_{50} value of 3,200 mg/m3, while the two alkanolamides, potassium soap, sodium sulfonate, and triazine had RD50 values of 100-200 mg/m^3. Thus, these latter 5 components were similar in potency as irritants. Based upon RD50 values, the proportion of each component in *Sample B*, and types of respiratory effects evoked in mice, we conclude that the two alkanolamides, potassium soap, sodium sulfonate, and triazine all contribute to the irritancy of *Sampie B*. This approach should be useful in the identification of irritants in other metalworking fluids. Supported by United Auto Workers and General Motors.

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AIRBORNE PARTICLES AND HEALTH: EFFECTS OF PM10 COMPONENTS ON THE RAT LUNG. M.T. Kleinman, D.K. Bhalla, W.J. Mautz and R.F. Phalen, Department of Community and Environmental Medicine, University of California, Irvine, CA 92717-1825.

Airborne particles less than 10 µm in diameter (PM10) are associated with significant adverse effects on human health including chronic lung diseases and mortality, but the mechanisms by which these particles cause or aggravate diseases are not specifically known. PM 10 particles represent a complex mixture, both in terms of size and chemical composition. Furthermore, the ambient aerosol composition varies markedly in different locations and at different times in the same location. Toxicological studies indicate that impairment of lung defenses may occur in rats exposed to components of the fine particle fraction of PM 10. Rats were exposed to purified air (control) or to two major fine particle (≤1 µm erodynamic diameter) components (Ammonium Nitrate, 350 µg/m^3 and Ammonium Sulfate, 70 µg/m^3) for a period of 8 weeks (4 hr per day, 4 days per week). Rats were also exposed to resuspended road dust (which is a major contributor to the course mode (2.5 µm) of PM10, at concentrations of 300 and 900 µg/m3 (4.0 µm mass median aerodynamic diameter). Exposure to the nitrate and sulfate aerosols caused significant increases in lung permeability and significant decreases in macrophage dependent lung defense functions (ability to engulf and destroy inhaled particles and pathogens). Significant alterations in lung morphometric characteristics also occurred. The road dust particles caused changes in macrophage functions only at the higher concentration and did not cause lung morphometric changes at either concentration. The results may provide some insight into the mechanisms of PM10-induced lung diseases. (Sponsored by California Air Resources Board Contract #A933-158).

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A 6 MONTH EPISODIC EXPOSURE OF RATS TO A MIXTURE OF OZONE, ACIDS, AND FINE PARTICLES OR TO THE OZONE COMPONENT ALONE. W.J. Mautz, M. T. Kleinman, D.K. Bhalla, and R.F. Phalen, Department of Community and Environmental Medicine, University of California, Irvine, CA 92717-1825.

Exposure to air contaminants usually involves inhalation of a mixture of materials. To determine whether the effects of ozone (O_3) exposure are substantially modified by acidic and particulate co-pollutants, groups of Fischer 344 rats were exposed nose-only 4 h/day, 3 consecutive days/week for 6 months to purified air; 0.3 ppm O_3; and to a mixture of 0.3 ppm O_3, 0.2 ppm NO_2, 0.1 mg/m^3 NH_4HSO_4, 0.05 mg/m^3 HNO_3, and 0.06 mg/m^3 carbon particles. The mixture and exposure protocol were based on air monitoring data in a heavily polluted region in California. At the end of 6 months exposure and 1 month following exposure, effects on several respiratory tract structural and functional measures were analyzed. Exposure to the mixture induced an elevation of breath frequency on the third days of episodes, and at end exposure, there was a depression of mucus secretory density in tracheal epithelium, increased numbers of mast cells in lobar bronchi, elevated cell proliferation in the terminal bronchioles and alveoli, increased permeability of the nasal epithelium, depression of pulmonary macrophage Fc receptor binding capacity, and a decrease in long-term tracer particle clearance rate. O_3 alone did not significantly alter these variables. Both the mixture and O_3 alone produced trends of change in morphometric measures of lung fine structure and in alterations of fixed lung volume. All of the significant effects of the mixture showed recovery at 1 month postexposure except for elevated mast cell numbers in lobar bronchi and cell proliferation in the alveolar zone. Trends of change in lung morphometric variables in the mixture and O_3 exposure were still present at 1 month post-exposure. These changes suggest that continued episodic exposure to the mixture can result in continued irritation of lung tissues and can compromise pulmonary defenses possibly leading to increased sensitivity to inhaled allergens and increased susceptibility to respiratory infections. Supported by California Air Resources Board No. A833-104.

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CHRONIC INHALATION EXPOSURE EFFECTS OF CUPRIC CHLORIDE ON RATS. V.V. Gabuchian, ICT College, 3200 Wilshire Blvd., Los Angeles, CA 90010; E.A. Babayan, Institute of General Hygiene and Occupational Diseases, 2 Acharian St., Yerevan 375040, ARMENIA; L.A. Saryan, West Allis Memorial Hospital Industrial Toxicology Laboratory, 8901 W. Lincoln Avenue, West Allis, WI 53227.

The effects of cupric chloride on reproductive function of white male rats, as well as the character and direction of the influence on the organism, were studied. Daily inhalation priming of rats with a concentration of 41.4±3.6 mg/m^3 led to the development of significant intoxication after three months. Besides external manifestations, signs of intoxication included morphofunctional alterations of certain organs and systems and serious changes of specific functions of the organism.

Among disturbances constituting the general response were growth delay of the animals and an increase in oxygen consumption. The total protein content and albumin and globulin fractions in blood serum were reduced. Activities of alkaline phosphatase, ceruloplasmin, and lactate dehydrogenase were altered. The content of uric acid increased in serum, the quantity of protein thiol groups decreased, and nonprotein thiol groups increased. Activity of alanine aminotransferase was depressed in serum. The disturbance of cardiovascular system activity, central hemodynamics, and metabolic processes of the myocardium were characterized by electrocardiographic and rheographic changes as well as by biological disturbances in the myocardium (increase of adenosine triphosphatase activity with a parallel decrease in the content of adenosine triphosphate, decrease of creatine phosphokinase activity, and an increase of creatine phosphate and inorganic phosphorus in the myocardium). The reproductive function of male rats suffered considerably. Morphofunctional changes of the testicles were accompanied by a decrease in mass of the testicles and the epididymis, and a decrease of osmotic resistivity and mobility time and an increase in the quantity of dead and deformed spermatozoa There was an abrupt decrease in testosterone and estradiol in blood.

After four months exposure at a concentration of 5.2±0.4 mg/m^3, signs of general toxic influence were practically absent. However, the indices characterizing the reproductive function of male rats continued to differ from those of the control group. Though the disturbances were less impressive, some (quantity of dead and deformed spermatozoa, osmotic resistivity) differed considerably from the indices of the control animals and had statistical significance.

The disturbances revealed in rats chronically exposed to a concentration of 5.2 mg/m^3 of cupric chloride were identified as specific threshold effects. Taking into account this threshold and choosing 10 as the coefficient of safety, the concentration limit of cupric chloride in working zone air was established at the level of 0.5 mg/m^3.

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AN EVALUATION OF THE ADEQUACY OF THE THRESHOLD LIMIT VALUE FOR CYCLONITE. P.L. Williams, Environmental Health Science, 201 Dairy Science Building, University of Georgia, Athens, GA 30602-2102; R.C. James and S.M. Roberts, Center for Environmental and Human Toxicology, University of Florida, One Progress Blvd., Box 17, Alachua, FL 32615.

Cyclonite (or RDX) is an explosive material that is commonly compounded with 2,4,6-trinitrotoluene (TNT). The current American Conference of Governmental Industrial Hygienists (ACGIH) 8-hour, time-weighted average (TWA) Threshold Limit Value (TLV) for cyclonite is 1.5 mg/m^3 and it contains a "Skin" notation. This TLV originally was derived based on an analogy between RDX and TNT; however, TNT TLV's has been lowered to 0.5 mg/m^3. The present study has reviewed the human and ani-

(400 ppm) for days 1–20 and 15–20 respectively, both single and in combination. The female liver weight ranged from 0.592 to 1.047 g, while the males ranged from 0.716 to 1.043 g. Aroclor 1254 significantly increased the liver weight of males and females over the controls. Interestingly, the liver weight of the males exposed to Cd-alone (0.716 g) was significantly lower than males and females exposed to PCB-alone 1.043 g and 1.047 g respectively, as well as, males exposed to PCB-Cd in combination. However, PCB-Cd group seem to reflect the toxicity of the PCB exposure. While the liver weights of the male and female controls were not significantly different, the females exposed to the combination of PCB-Cd was significantly lower than males in the same treatment group, as well as females exposed to PCB-alone. Unlike the males, the PCB-Cd combination in the females seem to reflect the toxicity of Cd. Preliminary indication of cytochrome P450 and porphyrin profiles are in progress and suggest that further research is needed to characterize the toxicity of environmental contaminants both single and in combination in males and females. This work was supported by NIH, NIGMS grants GM08117, GM08387 and NIEHS ES04696.

225 DYNAMIC ORGAN CULTURE TO ASSESS THE TOXICITY OF CHROMIUM/HALOGENATED HYDROCARBON MIXTURES

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Perchloroethylene (PCE), trichloroethylene (TCE) dichloroethylene (1,1-DCE), and chromium are common groundwater contaminants. Precision cut rat liver slices were used to evaluate the relative toxicities of the halogenated hydrocarbons in the presence of chromium containing compounds (Cr^{+3}, Cr^{+6}). The relative uptake of each form of chromium was evaluated using ^{51}Cr. Uptake of chromate (CrO_4^{-2}) via the sulfate anion transporter was assessed. Liver slice viability was measured by quantitating potassium content and leakage of lactate dehydrogenase (LDH) into the medium. Slices were exposed to gas phase concentrations of 2.5–100 ul halogenated hydrocarbon/L and 1×10^{-7} M–5×10^{-3} M sodium chromate or chromium chloride in the medium. Sodium chromate (Cr^{+6}) showed additive toxicity when present in the medium of slices exposed to TCE, DCE or PCE. Conversely, chromium chloride exhibited some protection against toxicity when administered in the medium of slices exposed to TCE, DCE or PCE. Uptake of ^{51}Cr as sodium chromate was not increased by addition of TCE, DCE, or PCE. This is contrary to our previous *in vivo* results. Minimal uptake of $^{51}CrCl_3$ was demonstrated in the presence *or* absence of TCE, DCE or PCE. Increasing the concentration of sulfate anion in the medium inhibited chromate uptake. Addition of 0.5 mM 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS), an inhibitor of anion transporters, reduced chromium uptake by nearly 50%. These results imply that chromate can enter cells via anion transport. TCE and DCE reduced the viability of rat liver slices in a dose responsive manner with DCE exerting its toxic effect at lower concentrations than TCE. However, PCE showed an interesting dose response in liver slices. It was less toxic than DCE or TCE until a 'threshold' concentration (≈50–70 ul PCE/L in 95% O_2/5% CO_2) where it became more toxic than either of the other halogenated hydrocarbons. This response may reflect depletion of a critical cofactor such as glutathione at the threshold concentration. (AHA AZ Affiliate #AZG-12-93; NIEHS #NIH ES-04940, The University of Arizona Undergraduate Research Grant Program.)

226 EVALUATION OF RESPIRATORY IRRITATION OF A METALWORKING FLUID (MWF) VS. ITS INDIVIDUAL COMPONENTS USING MICE

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Currently, there is a renewed interest in defining human health effects from exposure to MWFs. Some health-related concerns are due to the change from traditional "cutting oils" to newer types of MWFs (synthetics, semi-synthetics, solubles) which are complex chemical mixtures. Little toxicological information is available on newer MWFs or on components used in these fluids. The present study focused on the respiratory irritancy of individual components used in MWFs. Specifically, this study was based on previous results with aerosolized MWF "A" which was a synthetic fluid with 11 components. It evoked sensory and pulmonary irritation in mice and had an RD_{50} (concentration evoking a 50% decrease in mean respiratory frequency, *f*) of 119 mg/m^3 (Schaper and Detwiler, Fundam.Appl.Toxicol. 16, 309–319, 1991). In the present study, mice were exposed for 3 hr to aerosols of each component in MWF "A". Sensory and pulmonary irritation were evoked by each component, except a defoamer. For the 10 "active" components, concentration-response relationships (using *f*) and RD_{50} values were obtained. The 3 most potent components were tolutriazole, a preservative, and fatty acid alkanolamide condensates; all had RD_{50} values similar to that for MWF "A". It was concluded that these 3 contributed to the respiratory irritancy of MWF "A". This approach should be useful to identify irritants in other MWFs. Supported by UAW-GM.

227 HOST RESISTANCE TO BACTERIAL, VIRAL, OR TUMOR CELL CHALLENGE FOLLOWING EXPOSURE TO WOOD STOVE (WS) AND OIL FURNACE (OF) EMISSIONS

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Increasing use of WSs to supplement conventional heating systems has had an impact on air quality. WSs emit a complex mix of CO, SO_2, NO_x's, and organic compounds, whereas OF generally emit fewer constituents at lower concentrations. Effects of emissions from these sources on several host resistance models were compared. Mice received a single 6 hr exposure to emissions and were challenged immediately thereafter by aerosol with *Streptococcus zooepidemicus*. Mortality was enhanced in mice exposed to WS but not in those exposed to OF emissions. Mice also were exposed 6 h/day for 4 days and challenged after the 2nd exposure with influenza virus or B16 melanoma tumor cells. Neither OF nor WS emissions affected mortality, virus titers, or lung wet weight following influenza infection. However, significantly more B16 tumors were detected in lungs of mice exposed to WS emissions. More tumors were also seen in mice exposed to OF emissions although the difference was not statistically significant. Results indicate that WS emissions enhance susceptibility of mice to both bacterial and tumor cell challenge, whereas OF emissions had little or no effect. (This abstract does not reflect EPA policy.)

228 COMPARATIVE AND PREDICTIVE ACUTE TOXICITY AND PRIMARY SKIN IRRITANCY OF CRUDE ACETALDEHYDE SAMPLES (CONTAINING >2% ACROLEIN AND <2% ACROLEIN)

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The acute toxicity and primary skin irritancy of crude acetaldehyde (CA), an industrial by-product, were evaluated. Acrolein content in CA varies; therefore, 2 representative samples (containing 3.1% or 1.7% acrolein) were tested and the results compared. Perorally, the CA-3.1% had an LD50 of 297 mg/kg for both male and female rats, while the CA-1.7% had LD50s of 595 and 682 mg/kg for males and females, respectively. By the cutaneous route (female rabbits), the LD50 for the CA-3.1% was 933 mg/kg; that for the CA-1.7% was 771 mg/kg. For both materials, moderate to severe skin irritation (including superficial to full-thickness necrosis) resulted after a 4-hour application of 0.5 ml to occluded rabbit skin. As expected, doubling the acrolein content in CA essentially doubled the peroral toxicity. However, the predicted peroral toxicity of CA, calculated from reported LD50s for acetaldehyde (1930 mg/kg) and acrolein (46 mg/kg), was considerably less than observed in these studies (based on both additive and individual toxicity from the 2 components). Conversely, prediction of individual component toxicity from the observed CA results greatly overestimated their toxicity. It was concluded that the peroral toxicity of CA was largely related to acrolein toxicity, but that the toxic effects were more than additive (synergistic). Although clinical signs of peroral toxicity were similar for both CA samples, the CA-3.1% produced more rapid death. Cutaneous toxicity and irritancy were similar for both samples, perhaps indicating that volatility of the samples limited the amount of absorption.

229 THE EFFECTS OF JP-8 JET FUEL ON SPRAGUE-DAWLEY MALE RATS AFTER A 90-DAY EXPOSURE BY ORAL GAVAGE

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The U.S. Air Force is converting from JP-4 jet fuel to a less volatile JP-8 jet fuel similar to commercial Jet A. A 90-day inhalation study with JP-8 vapor conducted by this laboratory using F-344 rats and C57BL/6 mice resulted in no treatment-related adverse effects except for the alpha 2-microglobin nephropathy in male rats. In the present study male rats were dosed with neat JP-8 (0, 750, 1500, 3000 mg/kg) daily by gavage for 90 days in an effort to further characterize the kidney lesion and assess any additional adverse effects which may be associated with the prolonged exposure to this fuel. Results of this study revealed a significant dose dependent decrease in body weights of

trol group. It is concluded that the ventilation system combined with the use of PPE contributed to preventing undesired changes in respiratory function. However, improvements in the ventilation system may be necessary to further reduce the exposure to air-contaminants in the event the PPE fail to provide supplementary protection.

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EVALUATION OF RESPIRATORY SENSITIZATION TO THERMAL DECOMPOSITION PRODUCERS (TDP) FROM PLYPROPYLENE HOMOPOLYMER (HP) IN GUINEA PIGS. M.M. Schaper and K.A. Detwiler-Okabayshi, University of Pittsburgh, Graduate School of Public Health, Center for Environmental and Occupational Health and Toxicology, 260 Kappa Drive, Pittsburgh, PA 15238.

Recently, there was a case report suggesting respiratory sensitization of a worker exposed to TDP from HP. To evaluate this potential effect, groups of guinea pigs were repeatedly exposed to TDP from HP. In the first experiment, a mass of 16 grams of HP was heated isothermally at 285° for 30 minutes/day x 5 consecutive days. Some sensory irritation and coughing occurred in these exposures, but the main effect was acute bronchoconstriction. As a result of intense bronchoconstriction, 2/4 animals died by the end of the 5th exposure, and no further exposures to TDP were conducted with these animals. Subsequently, other experiments were conducted in which groups of guinea pigs were exposed for 30 minutes/day x 5 consecutive days (i.e., Day 1,2,3,4,5) to TDP from 1.0, 0.1, or 0.03 gram of HP, heated isothermally at 285C. There was some sensory irritation and coughing in exposed animals, particularly during heating of 1.0 gram of HP. No bronchoconstriction occurred, and no animals died during the repeated exposures to 1.0,0.1, or 0.03 grams of HP. A 2-week rest period then followed these 5 exposures. On days 15, 29, 43, 57, and 71, each group of guinea pigs was "challenged" with the TDP, using the same mass of HP employed on days 105. As in the first 5 exposures, only sensory irritation and coughing were evoked in the animals. There was no evidence of rapid, shallow breathing, often seen at the start of a reaction to a respiratory sensitizer, and no bronchoconstriction, which would be expected as the reaction intensified. Thus, the results suggested that the TDP from HP did not produce respiratory sensitization in guinea pigs. Supported by UAW-GM.

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PREDICTION OF AN OCCUPATIONAL EXPOSURE LIMIT FOR A METALWORKING FLUID (MTF) ON THE BASIS OF ITS INDIVIDUAL COMPONENTS. S.P. Krystofiak, K.A. Detwiler-Okabayashi, M.M. Schaper, University of Pittsburgh, Graduate School of Public Health, Center for Environmental and Occupational Health and Toxicology, 260 Kappa Drive, Pittsburgh, PA 15238.

The potential human health effects from exposure to MWFs are not fully understood and there has been recent debate regarding an acceptable exposure limit for workers exposed to MWFs. The Threshold Limit Value (TLV) proposed by the American Conference of Governmental Industrial Hygienists (ACGIH) for mineral oil mist is 5 mg/m^3. However, newer types of MWFs (synthetics, semi-synthetics) contain little or no oil, and it is unclear what the exposure limit should be for these fluids. In the present study, a mouse bioassay was used to evaluate the sensory and pulmonary irritating effects of MWF "B" (semi-synthetic). On the basis of mouse data, an exposure limit of 2.6mg/m^3 was proposed for MWF "B" to protect workers from its sensory and pulmonary irritating effects. The mouse bioassay was also used to evaluate the irritancy of the 7 major components of MWF "B". The ACGIH formula for mixtures was then applied, and by incorporating the mouse data from the 7 components, an exposure limit of 5.0 mg/m^3 was obtained for MWF "B". Thus, there was reasonable agreement between the 2 exposure limits for MWF "B". Either approach should be acceptable for predicting an exposure limit for MWF, but it may be advantageous to identify components that are particularly irritating. With this information, MWFs could be reformulated, replacing such components. This study did not suggest that the TLV for all MWFs should be 2.6 mg/m^3 or remain at 5.0 mg/m^3. Indeed, if there is to be a single TLV for all MWFs, a more conservative value may be necessary. Additional data should provide better direction for resolving this issue. Supported by UAW-GM.

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EXPOSURE TO ZINC DI-2-ETHYLHEXYL DITHIOPHOSPHATE, A POTENTIAL REPRODUCTIVE HAZARD AT A LUBE OIL ADDITIVES PLANT. S.J. Stevenson, Chevron Chemical Co., P.O. Box 70, Belle Chasse, LA 70037; M.A. Al-Safwani, Saudi Aramco, P.O. Box 2609, Dhahran 31311, Saudi Arabia; R.T. Cheng, Chevron Research & Technology, P.O. Box 4054 Richmond, CA 94804.

As part of a voluntary initiative to generate toxicity data on high production volume chemicals, the Chemical Manufacturers Association (CMA) Petroleum Additives Group sponsored an animal study on zinc di-2-ethylhexyl dithiophosphate (ZDTP). In 1994, based on preliminary testing results, CMA notified the Environmental Protection Agency under Section 8(e) of TSCA that ZDTP may be a reproductive and development hazard. ZDTP is used as an antioxidant in lube oil for industrial and automotive engines.

An assessment of worker exposures to ZDTP was initiated at a lube oil additives plant following the EPA notification. A walk-through survey of the manufacturing process was conducted beginning at the station where zinc (as zinc oxide) was added to the process and ending at the waste material disposal area where ZDTP-containing filter cake was dried out and incinerated. Tasks where workers could be exposed to ZDTP were identified and priority-tanked. Because ZDTP is a viscous liquid with minimal vapor pressure, controlled experiments were run to confirm that airborne ZDTP can be collected with a personal air sampling pump and a particulate filter. The air samples were analyzed for zinc by atomic absorption spectrometry method to determine ZDTP concentrations.

While waiting for additional toxicological research to generate more definitive dose-response findings, an interim exposure guideline for ZDTP was set at 1.0 mg/m^3 (or 0.08 mg/m^3 as zinc). Monitoring results indicate that the worst-case exposure was 0.3 mg/m^3 of ZDTP, about one-third of the interim exposure guideline.

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DIOXIN TOXICITY AND EXPOSURE: A SURROGATE FOR ALL CHLORINATED ORGANIC COMPOUNDS? S.W. Pirages, C.L. Richard, Risk Communication International, 7910 Woodmont Ave., Suite 700, Bethesda, MD 20814.

A draft of the U.S. Environmental Protection Agency's dioxin reassessment report was released in September 1994. The report represents an in-depth analysis of toxicity and potential for exposure to the general population from dioxin-like compounds (e.g., dioxin, furan and PCB). Recent calls to ban chlorine have used PCB and DDT properties and toxicities as a justification. Because this EPA report provides the most extensive review of any chlorinated organic, dioxin may become the new surrogate.

Extensive uncertainties are associated with data and conclusions reached in the reassessment report. These uncertainties include inconsistencies between animal and human toxicity studies, use of untested methodologies for comparing animal and human body burdens, limited data about potential sources of dioxin-like compounds, and use of risk assessment methodologies that have not been validated. A discussion of the influence of these uncertainties on any conclusions about exposure and toxicity of dioxin-like compounds.

Chlorinated compounds comprise a broad range of products that contribute to a high quality of life and a high standard of health care. Given the diversity of chlorinated organics, dioxin-like compounds should not be used as surrogates for an evaluation of all chlorinated organics; nor for a decision concerning the need to restrict chlorine use. The presence of a chlorine atom has limited, if any, influence on hazardous properties of a compound; examples illustrating this point are discussed. The concept of toxicity persistence and bioacummulative ability is a more appropriate approac for evaluating the potential harm to public health and the environment posed by any compound.

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EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO P-DIOXIN (TCDD) ON CYCLICITY AND OVULATION IN FEMALE SPRAGUE-DAWLEY RATS. X. Li[1], D.C. Johnson[2], and K.K. Rozman,[1,3] [1]Department of Pharmacology, Toxicology and Therapeutics, [2]Department of Obstetric and Gynecology, University of Kansas Medical Center, Kansas City, KS 66160 (USA and [3]Section of Environmental Toxicology, GSF-Institut fü Toxikologie, 85758 Neuherberg, Fed. Rep. Germany.

TCDD has unwanted contaminant produced during the manufacture of chlorphenols, hexachlorphene, and phenoxy hervicides. More recently the major source of TCDD reaching the environment is municipal waste in cineration and some industries in many countries. TCDD is one of the most toxic chemicals in some mammalian species. Recently increasing attention has been paid to reproductive and developmental toxicity of TCDD and related compounds, particularly in male populations. However, the effects of TCDD on the female reproductive system have not been investigated i detail. The present study was undertaken to determine the acute affect of a single dose of TCDD on the estrou cycle and ovulatory function in adult Sprague-Dawley rats. RCDD at the dose of 10 µg/kg resulted in irregularity of cycles, characterized mainly by prolonged perio of diestrus. In TCDD-treated rats with a normal estrou cycle, the ovulatory rate and the number of ova recovered were significantly reduces. The results also clearl show that derailment of cyclicity is associated with a r duction in ovulation. The data further suggest that TCDD alters reproductive function by a direct and an i direct action on the ovary and the hypothalamic-pituitary axis, respectively. Further evidence for TCDD involvement in the two systems is exemplified by reduced ovulation in immature rats with an exogenously induced estrous cycle.

▼ Papers 74–83 Air Sampling Instrument Performance

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HEALTH RELATED SAMPLING STRATEGIES IN THE WORKPLACE: RELEVANCE TO THE ENVIRONMENT. R.J. Aitken, A.M. Johnston, Institute o Occupational Medicine, 8 Roxburgh Place, Edinburgh, EH8 9SU, UK.

There is now widespread agreement that the sampling of aerosols in the workplace should be based on particle size-selective criteria. Definitions for these particle size-selective criteria, the inhalable, thoracic and respirable conventions, have now been agreed on internationally by the American Conference of Governmental Industrial Hygienists (ACGIH), International Standards Organization (ISO) and the European Committee of Standardization (CEN). The rationale for this approach is clear in that the purpose of sampling is to provide a determination of the amount of airborne material which may penetrate to and therefore damage the target area of the respiratory tract. Sampling of airborn material in the environment has been primarily concerned with issues such as emission monitoring impairment and visibility. The two principal sampling conventions for environmental sampling are the PM10

92 ACUTE INHALATION TOXICITY OF CARBONYL SULFIDE

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Carbonyl sulfide (COS), present in several commodity fuels including propane and synthesis gas, is rapidly metabolized to H_2S in the body. The purpose of this research was to determine the effects of exposure concentration and time on the toxicity of acutely inhaled COS in F344 rats and to compare the toxic effects with those of H_2S. Groups of male and female rats were exposed by nose-only inhalation to 250 – 1400 ppm COS for up to 4 hr and observed for up to 14 days. No lethality was observed below 500 ppm; the median lethal concentration was estimated at 590 ppm. Time to death was 30 – 60 min from the start of the exposure at 1400 ppm, 2 – 3 hr at 1000 ppm, 3 – 4 hr at 750 ppm, and > 4 hr at 590 ppm. Exposure to the higher concentrations caused a phase of excitation followed by a period of depression in which heart rate and body temperature were reduced. Gross pathological changes were minimal in rats inhaling 1400 ppm; most body organs, including lung, liver, kidney and GI tract were severely congested in rats inhaling 750 – 1000 ppm. Rats surviving exposure to > 500 ppm COS had motor impairment consistent with damage to the vestibular-cerebellarstriatal pathways. Preliminary histopathology showed swelling of myelin sheaths in the corpus callosum, cerebellum, and pyramidal tract. Partial-to-complete resolution of the gross pathological and behavioral changes occurred during the 14-day post exposure observation period. [Research sponsored by the Office of Environmental Restoration, U.S. Department of Energy under Contract No. DE-AC04-76EV01013.]

93 *IN VITRO* EFFECTS OF DUSTS ON PULMONARY CELLS USING MACROPHAGE AND EPITHELIAL CELLS IN CO-CULTURE

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The current studies were designed to evaluate two pulmonary cell-types, i.e., rat lung epithelial (L2) cells and rat alveolar macrophages (AM) for *in vitro* studies with particulates and to correlate the results of *in vitro* studies with data generated from inhalation studies in rats using the same particle-types. L2 cells, AMs, or both cell-types in combination (i.e., co-cultured) were incubated at various concentrations with zinc oxide (ZnO) particles or titanium dioxide (TiO_2) particles. Cytotoxicity was assessed using the MTT assay, as well as by measuring for lactate dehydrogenase activity in the culture fluid. The data obtained form the *in vitro* assays were then compared to the effects measured following 6 hour or 3-day exposures to each of these dusts.
The results of *in vitro* studies demonstrated that ZnO particles were extremely toxic to L2 cells but had little effect on AMs. When the two cell-types were co-cultured, AMs could not protect the toxic effects of ZnO to L2 cells. In contrast, TiO_2 particles at 3 doses had no significant effect upon either L2 cells or AMs. Following a 1 or 3-day inhalation exposure to ZnO particles at concentrations ranging from 10 to 100 mg/m^3 or to TiO_2 particles at 100 or 250 mg/m3, rats were sacrificed at sequential postexposure time periods and their lungs lavaged. Inhalation of ZnO caused a potent but short-lived pulmonary inflammatory response, suggesting rapid clearance. Three-day exposures to TiO_2 particles at 100 mg/m^3 produced a short-lived neutrophilic response without corresponding BAL parameters of inflammation. However, 3-day exposures to TiO_2 at overload concentrations (250 mg/m^3) produced transient inflammatory responses in the absence of continuing exposure. The results of this study demonstrate the need for better validation in carrying out *in vitro* studies with particles and fibers.

94 COMPARISON OF SENSORY AND PULMONARY IRRITATING EFFECTS OF AMINES FOUND IN METALWORKING FLUIDS (MWF)

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In this study, a mouse bioassay was used to assess the sensory and pulmonary irritation of amines found in MWF: 3 ethanolamines (mono-, di-, tri-) and 3 isopropanolamines (mono-, di-, tri-). Each amine was aerosolized for 3 hours, during which time the respiratory pattern and frequency (f) of mice were monitored. Sensory irritation (S) occurred rapidly upon exposure to each amine. With the isopropanol-amines, pulmonary irritation (P) also occurred later in the exposures. As a result of S and/or P, f decreased. Using f, a concentration-response relationship was developed for each amine. From such relationships, the concentration producing a 50% decrease in f due to S or P was determined ($RD_{50}S$ or $RD_{50}P$). $RD_{50}S$ values for the ethanolamines ranged from 500–1500 mg/m^3 while $RD_{50}P$ values for the isopropanolamines ranged from 400–4,000 mg/m^3. Within the series of ethanolamines, di- and tri- were similar in (S) potency to one another and both were more potent than mono-. Within the series of isopropanolamines, mono- and tri- were similar in (P) potency to one another and both were more potent than di-. These toxicological data may be helpful in selecting amines to formulate MWF. Also, the respiratory irritancy of amines and other components found in MWF may be evaluated with the mouse bioassay.

95 URINARY METABOLITES OF RESIN ACIDS IN PERSONS EXPOSED TO AIRBORNE FIBERS FROM A MEDIUM-DENSITY FIBERBOARD FACTORY

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Individuals living in the immediate vicinity of a medium-density fiberboard factory were documented by video and site collections to be regularly exposed to fallout of fiber coming as airborne emissions from cyclones used in the manufacturing process which utilized pine wood chips, urea, and urea-formaldehyde resin. Fibers had been demonstrated by others to be of both respirable and non-respirable size and to contain crystals of the urea-formaldehyde resin. Fiber obtained from a production line inside the factory, as well as, from the property of the exposed individuals was submitted to GC/MS analysis and found to contain several different classes of chemicals: fatty and dicarboxylic acids; monoglycerides of fatty acids; various aromatic aldehydes, acids, esters, alcohols, phenols, and amides; various resin acids and their oxidized derivatives; indoles; and phthalates. Dehydroabietic (I) and abietic (II) acids were principal components. Urine specimens were obtained from the individuals approximately 4 hrs. following a period of documented airborne fiber emissions. GC/MS analysis of these specimens demonstrated the presence of tetrahydroabietic acid (III), 7-ketodehydroabietic acid (IV), and 7-hydroxyabietic acid (V). The presence of IV and V were believed to be due to biotransformation of I and II, respectively, whereas, the presence of III may be due to disproportionation of II during the manufacturing process. These findings coupled with the finding of wood fibers in the sputum of these individuals by others, suggested that the route of entry for these chemicals was *via* respiration of the treated-wood fibers.

96 OLFACTORY EPITHELIUM AND LIVER PEROXISOME PROLIFERATION EVALUATIONS AFTER INHALATION EXPOSURE TO METHYLETHYLKETOXIME

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Male CD-1 mice were exposed 6 hrs/day, 5 days/week for 1, 2, 4 or 13 weeks via whole-body inhalation exposures to methylethylketoxime vapors at 0, 3, 10, 30, or 100 ppm (10/group/interval). Satellite animals were removed after 1, 2, 4 or 13 weeks of exposure and allowed to recover for 4 or 13 weeks (5/group/interval). Nasal turbinates were evaluated microscopically and nasal maps of the lesions prepared. Dose related degeneration of olfactory epithelium lining the dorsal meatus was seen in the anterior region of the nasal cavity, not laterally or posteriorly. Degeneration was present after 1 week but the incidence and severity did not increase with further exposure. The effect was reversible; recovery was complete within 4 weeks at 10 ppm and nearly complete within 13 weeks at 30 ppm. Three ppm was considered to be a NOEL. Using electron microscopy and palmitoyl-CoA oxidation activity evaluations, there was no evidence of any hepatic peroxisome proliferation. Exposure to 30 and 100 ppm however did produce significant increases in levels of hepatic non-protein sulphydryl groups (primarily reduced glutathione).

97 REFORMULATED GASOLINE EXPOSURE ASSESSMENT IN SOUTHEAST WISCONSIN

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The Clean Air Act Amendments (CAAA) of 1990 required that severe ozone nonattainment areas, including the Milwaukee metropolitan area, sell exclu-

In The Industrial Metalworking Environment -- Assessment and Control: Proceedings of the Symposium in Dearborn, Michigan, November 13-16, 1995, by the American Automobile Manufacturers Association. Washington, D.C.: AAMA, 1996, pp.88-94.

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

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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* ($RD_{50}P$) was obtained for each MWF and component. By dividing the $RD_{50}P$ values by 60, occupational exposure limits (OELs) were suggested. These limits, which were generally in the range of 2-10 mg/m^3, 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)[1]. 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)[2].

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 μm may be formed. In some instances, the particle (droplet) size may be even less than 1 μm[3,4]. 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[5,6]. 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[7-15].

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[16-22]. 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_{50}[16,18,23]. In the original definition, this decrease in *f* was due to *sensory irritation*. Recently a descriptor, "S" or "P", has been added after RD_{50} to designate whether the decrease in *f* (at the time of maximum response) is predominantly due to sensory or pulmonary irritation[13-15]. Thus, $RD_{50}S$ or $RD_{50}P$ 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 $RD_{50}S$, would experience intolerable burning of the eyes, nose, and throat[18,23]. To prevent these effects in occupational settings, an acceptable exposure level may be predicted by dividing the $RD_{50}S$ by 33 (or 0.03 x $RD_{50}S$). Indeed, an excellent correlation has been demonstrated for 89 industrial chemicals between their Threshold Limit Values ($TLVs^{TM}$)[5] and 0.03 x $RD_{50}S$ values[18,23]. For pulmonary irritants, such a relationship between the TLVs and $RD_{50}P$ values has not been established. It has been suggested that the $RD_{50}P$ be divided by 60 to arrive at a concentration that would prevent pulmonary irritation in workers[24,25]. Because pulmonary irritants may produce hemorrhage and edema in the lung, and even death[16], 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[7-15,17] 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[11,15]. 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[7-15].

A Marple personal cascade impactor was used for sizing the aerosols[7-15]. For the majority of MWFs and components evaluated in this study, the mass median aerodynamic diameter (MMAD) was 1-2 μm and the geometric standard deviation (σ_g) was appproximately 2[7-15].

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* ($RD_{50}P$) 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 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 2-10 mg/m^3. Also listed in Table 1 are 6 industrial chemicals whose *sensory* irritating properties have been well-established[18,23,26]. 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[26]. 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 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 2-10 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^3 Permissible Exposure Limit (PEL)[6] or Threshold Limit Value (TLV)[5] is inappropriate. Thus, our proposed limits of 2-10 mg/m^3 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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Table 1. Comparison of RD_{50} Values for the Seven MWFs[1] Studied Vs. Those for Known Irritants[2]

Chemical or Mixture	$RD_{50}P$ (or $RD_{50}TC$)[3] (mg/m^3)	Occupational Exposure Limit[4] (mg/m^3)
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)	10.80 (5.7 ppm)
Hydrogen Chloride	806 (540 ppm)	13.40 (9.0 ppm)
Sulfur Dioxide	996 (380 ppm)	16.60 (6.3 ppm)
Ammonia	1115 (1603 ppm)	18.60 (26.7 ppm)

[1]From Reference 7.
[2]From Reference 26.
[3]$RD_{50}TC$ refers to the concentration capable of producing a 50% decrease in *f* in tracheally-cannulated mice.
[4]Each RD_{50} value was divided by 60 to suggest an occupational exposure limit that would prevent *pulmonary irritation* in workers.

Table 2. RD_{50} Values for Components in MWFs A, B, and E[1]

MWF	Component	$RD_{50}P$ (mg/m^3)	Occupational Exposure Limit[2] (mg/m^3)
E	Sodium Sulfonate	102	1.7
B	Sodium Sulfonate	103	1.7
E	Tall Oil Fatty Acids	105[3]	3.2[3]
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

[1]From References 9-15.
[2]Each $RD_{50}P$ value was divided by 60 to suggest an occupational exposure limit that would prevent *pulmonary irritation* in workers.
[3]The $RD_{50}S$ value for the tall oil fatty acids was divided by 33 to suggest an occupational exposure limit that would prevent *sensory irritation* in workers.

KEY WORDS:

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

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1. Metalworking fluid
2. Mouse bioassay
3. Sensory irritation
4. Pulmonary irritation
5. Respiratory frequency
6. Concentration-response relationship
7. RD_{50}
8. Occupational exposure limit

ORIGINAL INVESTIGATION

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An attempt to define a just detectable effect for airborne chemicals on the respiratory tract in mice

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Abstract We have attempted to define a just detectable effect (JDE) for three different types of reactions along the respiratory tract: (a) sensory irritation of the upper airways (S), (b) airflow limitation along the conducting airways (A), and (c) pulmonary irritation at the alveolar level (P1 or P). Each type of reaction, S, A, P1 or P, was recognized by analyzing the breathing pattern of unanesthetized mice held in body plethysmographs. A rule-based computer program analyzed each breath during a period of 3.75 h and classified each breath as normal (N) or falling in any of the above categories (i.e., S, A, P1 or P). Eight groups of four mice were used for sham exposures: exposed to water vapor. These data sets were used, as sham exposure data, to define the variation which can occur with time in order to define an expected range of normal variation. Once this range was established, we defined JDE values for each type of effect and used such values to evaluate the results obtained in exposed animals. Eight groups of four mice were exposed to a mixture of airborne chemicals, machining fluid G (MFG), at concentrations from 0.17 to 55 mg/m^3. Data sets for individual animals and for each group of animals exposed to MFG were analyzed to determine if and when a particular effect occurred. It was possible to recognize the effects of low exposure concentrations on groups of exposed animals or individual animals within each group. This procedure will be valuable when investigating the effect of airborne chemicals and when it is impossible to generate high exposure concentrations to define concentration-response relationships.

Key words Sensory irritation · Pulmonary irritation · Airflow limitation · Machining fluids · Metal working fluids · Toxicity of mixtures

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Introduction

In two previous articles (Vijayaraghavan et al. 1993, 1994), we introduced the rationale to recognize three types of effects which can occur along the respiratory tract in mice: (a) sensory irritation of the upper respiratory tract (S), (b) airflow limitation along the conducting airways (A) and (c) pulmonary irritation at the alveolar level (P). We showed that these effects induced characteristic modifications of the normal breathing pattern of unanesthetized mice and that rule-based computer programs can classify each breath as either normal (N) or S, A or P as well as the combinations S + A, P + A, P + S and P + S + A. In a later article (Boylstein et al. 1995b), one additional breath classification, the first phase of pulmonary irritation (P1), was added as this may also occur in mice, prior to the more intense pulmonary reaction, P. A summary of this approach is presented in Table 1. Recently, we have used this approach to investigate a mixture of airborne chemicals, machining fluid G (MFG) (Boylstein et al. 1995b). This mixture produced complex effects; in terms of exposure duration or exposure concentrations, and as to the types of reactions induced, principally S or P, but also A. Furthermore, we observed more variation in the reactions of individual animals within exposed groups than when investigating single airborne chemicals. In order to resolve and present these complex results, we used smoothing polynomial spline analysis of the time-series data sets obtained during these exposures (Boylstein et al. 1995b). It remains, however, that the lowest concentration evaluated was 70 mg/m^3, which is high in comparison to the current industrial exposure level (Threshold Limit Value, TLV), of 5 mg/m^3 (ACGIH 1994–1995). While MFG is primarily mineral oil (for which the above TLV is applicable), it also contains a number of other additives (Schaper and Detwiler 1991).

In order to evaluate lower exposure concentrations, we used the same smoothing polynomial spline analysis

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Table 1 Variables calculated by the computer program from measurement of airflow ($\dot{V}$) during inspiration and expiration ($\dot{V}I$, $\dot{V}E$) and their use in breathing pattern analysis for breath classification in categories other than normal (N)[a]

Variables used for abnormal breath classifications	Abb	Unit	Definition	Type of abnormal breath classification for which the variable(s) is used	Requirement for the variable for breath classification in comparison to $\bar{X}$ control value[b]
Time of braking	TB	s	Duration of the top of the VT wave	S: sensory irritation Basis: reflex reaction from stimulation of trigeminal or laryngeal nerve endings	Value for the breath is $> \bar{X} + 2SD$
Airflow at 0.5 VTE	$\dot{V}D$	ml/s	Airflow during expiration at 0.5 VT	A: airflow limitation Basis: lengthening of expiration to compensate for an increase in airflow resistance	Value for the breath is $< \bar{X} - 1.5SD$
Time of pause	TP	s	Duration of the bottom of the VT wave	P: pulmonary irritation Basis: reflex reaction from stimulation of pulmonary vagal type-C nerve endings	Value for the breath is $> \bar{X} + 2SD$
Tidal volume	VT	ml	Amount of air inhaled/ exhaled per breath	P1: pulmonary irritation, phase 1 Basis: same as P, low stimulation	Value for the breath is $< \bar{X} - 1.5SD$
Duration of inspiration	TI	s	Time from minimum to maximum VT	P1: pulmonary irritation, phase 1 Basis: same as P, low stimulation	Value for the breath is $< \bar{X} - 1.5SD$
Duration of expiration	TE	s	Time from maximum to minimum VT	P1: pulmonary irritation, phase 1 Basis: same as P, low stimulation	Value for the breath is $< \bar{X} - 1.5SD$
Respiratory frequency	BPM or f	–	Number of breaths occurring during a collection period of 14 s, converted to BPM	Not used but will increase during P1 decrease during S and A and decrease during P when TP is sufficiently large	

[a] Summarized from Vijayaraghavan et al. (1993, 1994) and Boylstein et al. (1995b)

[b] $\bar{X} \pm$ SD values for each animal are obtained for each variable, except BPM, by averaging the values for all the breaths during a baseline or control period of 15 min, approximately 3600 breaths. For the classification P1, three variables (VT, TI and TE) must decrease while for the classifications S, A or P only a single variable must increase or decrease. The combination classifications S + A, P + A, P + S and S + A + P are also made when two or more of the appropriate variables meet the criteria for each individual classification of the combinations. There is no possible combination with the classification P1 since a decrease in VT, TI and TE is not compatible with an increase in TB or TP or a decrease in $\dot{V}D$

approach to analyze data sets from groups of exposed animals as well as individual animals in each group. First, eight groups of four mice were used for sham exposures in order to establish a range within which the response for a group of exposed animals, and then for an individual animal, would be accepted as "within the expected normal variation range for sham exposures". From these sham exposure results, we present values which would be considered a "just detectable effect" (JDE) for each type of reactions: S, A, P1 or P. This is presented for groups of four exposed animals as well as for individual animals. The determination of a JDE is akin to defining the limit of detection for a particular analytical method. Thus the results presented here also serve to characterize the sensitivity of this bioassay as developed by Vijayaraghavan et al. (1993, 1994) and finalized by Boylstein et al. (1995b).

Materials and methods

Animals

Certified virus free (CVF) Swiss Webster male mice were received from Hilltop Lab Animals (Scottdale, Pa.) and were approximately 7 weeks of age upon receipt. They were held at least 1 week before being used for experiments. At the time of exposure, mice ranged from 25 to 30 g in body weight. They were held four per cage, in polypropylene cages, with corn pith as bedding material. Animals were provided food (Purina Chow) and water ad libitum.The animal room was maintained on a 12-h light/dark cycle. A total of 76 mice were used.

Machining fluid sample

MFG is a water soluble machining fluid containing four major components as described by Schaper and Detwiler (1991).

Aerosol generation and exposure groups

The aerosol of MFG was generated using a Pitt #1 glass generator, which for this fluid produced a particle size (Mass Median Aerodynamic Diameter) of 1.5 μm with a geometric standard deviation (σg) of 1.6 (Schaper and Detwiler 1991). Compressed air was passed into the generator at 21 psi (0.145 mPa) with the flow rate of MFG controlled by a Harvard apparatus syringe pump to establish the desired exposure concentrations as previously described (Schaper and Detwiler 1991; Vijayaraghavan et al. 1993). For sham exposures, deionized water was substituted for MFG. The exposure concentrations of MFG were obtained by gravimetric analysis and are ±10% as previously reported (Vijayaraghavan et al. 1993). The exposure concentrations were selected to be above and below what would be the TLV of 5 mg/m^3 for MFG, but below the lowest concentration of 70 mg/m^3 previously investigated (Boylstein et al. 1995b). A total of 11 exposure concentrations, some very closely related for those below 5 mg/m^3, were conducted: 0.17, 0.20, 1.25, 2.08, 2.30, 3.14, 3.42, 3.63, 13.7, 43.8 and 55 mg/m^3. A total of eight sham exposures, using deionized water to produce a single exposure concentration of 35 mg/m^3 of water vapor added to room air, were conducted. Each exposure group consisted of four naive animals.

Experimental protocol

All experiments lasted for a total of 235 min consisting of 15 min of baseline to obtain control data, 180 min for the exposure period and 30-min recovery period. Prior to initiation of the baseline period, mice were placed in the body plethysmographs and allowed to acclimate for approximately 15 min.

Exposure apparatus and airflow ($\dot{V}$) measurement

The apparatus consisted of a 1.5-l cylindrical glass exposure chamber to which four glass body plethysmographs were attached, as previously described (Vijayaraghavan et al. 1994). After placing the mice in plethysmographs, measurement of $\dot{V}$ was accomplished via a pneumotachograph to which a differential pressure transducer was attached. The analog signal from the pressure transducer was digitized and analyzed as previously described to obtain tidal volume (VT) and all the variables listed in Table 1, which were used to classify each breath (Vijayaraghavan et al. 1994; Boylstein et al. 1995b).

Computer programs for data collection and breath classification

The computer program has been previously described in detail (Vijayaraghavan et al. 1994; Boylstein et al. 1995b).

Briefly, the computer program used the digitization of $\dot{V}$ during inspiration ($\dot{V}$I) and expiration ($\dot{V}$E) to first calculate tidal volume (VT). It then calculated the other variables listed in Table 1, for each breath and for each animal, except for BPM which is the number of breaths obtained during a collection period of 15 s. While each collection period is for 15 s, data are actually collected for 14 s, with the computer program taking one s to reset prior to collecting data for the next period. These variables were then used to classify each breath as normal or into one of eight abnormal categories as shown in Table 1.

Data analysis, structures of data set

3120

The time-series data sets created for each experiment were as previously described in detail (Boylstein et al. 1995b). The first data set was for the six variables used for breath classification, as listed in Table 1. For each variable and for each animal, the mean values for all the breaths collected during each 15 s of each experiment were obtained, yielding a total of 780 data points (i.e., 15 min for baseline control data, plus 180 min of exposure data = 195 min × four collection periods of 15 s/min). These data sets for each animal were then combined for each experimental group of four animals to form a set containing ~~three, 120~~ data points which is called a "stacked set" (Boylstein et al. 1995b). Each variable was kept as a percentage of the control value; the control value being the mean value for all the breaths collected during the 15-min baseline period and made equal to 100 and is from a total of approximately 3600 breaths for each animal (Boylstein et al. 1995b). The second data set was identical to the first in terms of the number of data points, but was for the breath classifications. Here, for each 15-s period, a file was created containing the number of breaths in each classification, as a percentage of the total number of breaths accumulated during that period (Boylstein et al. 1995b). Again, one file was created for each animal and a "stacked set" was created for each exposure group.

Data analysis, statistical procedure used

The data sets for each variable, for individual animals and group responses (i.e., stacked sets) were analyzed via a computer program,

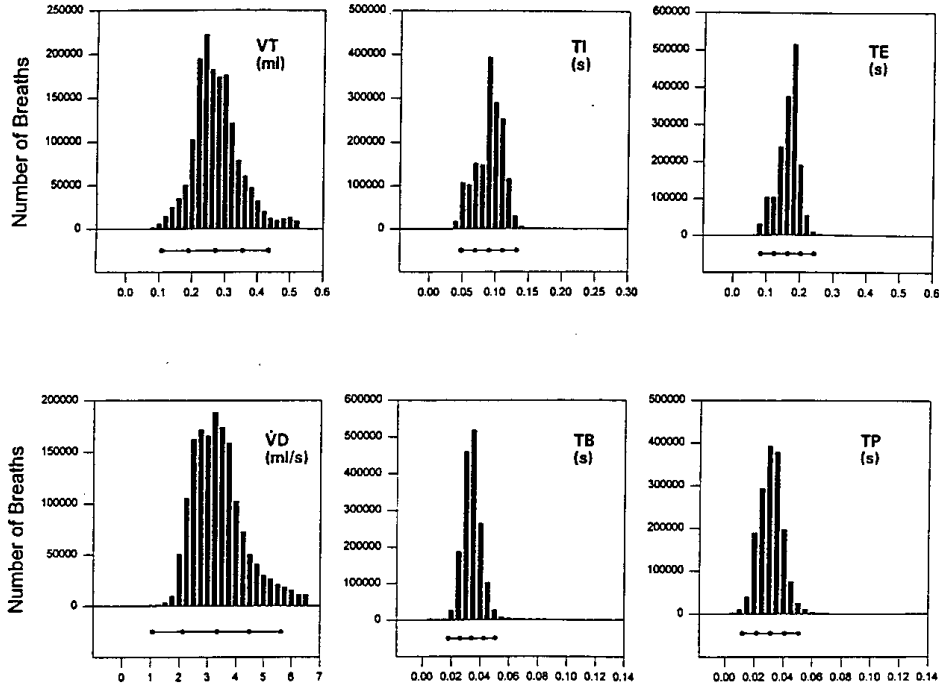


Fig. 1 Histograms of the six variables (VT, TI, TE, $\dot{V}D$, TB and TP) which are used in determining the classification of each breath as listed in Table 1. $n = 1\,620\,829$ breaths for each histogram. The line with dots beneath each histogram indicates the $\bar{X} \pm 1$ and ± 2 SD which are used by the computer program to classify each breath in categories other than normal (N), as described in Table 1

MLEGCV, using the maximum likelihood (ML) method to obtain a cubic smoothing polynomial spline curve, with 95% CI. The details of this program have been presented previously (Boylstein et al. 1995a, b). The same procedure was used for the breath classification data sets, after recurrence filtering (Boylstein et al. 1995b). Recurrence filtering was accomplished by inspecting each data set for consecutive breath classifications and replacing any abnormal classification by the classification N, unless there were three or more consecutive breaths classified in the same abnormal categories, as previously described (Boylstein et al. 1995b). This recurrence filtering helps reduce the number of abnormal breaths created by body movement of the unanesthetized animals in body plethysmographs which is unavoidable (Boylstein et al. 1995b).

Establishing a just detectable effect (JDE)

First, histograms for the six variables used for breath classification, as listed in Table 1, were prepared using all the breaths collected on all animals for all sham exposures ($n = 1\,620\,829$ breaths). The results (Fig. 1) showed that the SD multiples listed in Table 1 to classify breaths in categories other than N, as previously presented (Vijayaraghavan et al. 1994; Boylstein et al. 1995b) were still appropriate, in that more than 80% of the breaths will be classified as N during sham exposures. Then, the data sets for breath classifications for each sham exposure group ($n = 8$) and for each animal ($n = 32$) in these groups were processed via the MLEGCV program. The output of this program is a smoothed curve with 95% CI (examples are shown in Fig. 2 for groups and Fig. 5 for individual animals). From these plots, we searched the highest value reached above 0%, for each abnormal breath classification, either for a group or for an individual animal. We used the *higher* 95% CI smoothed curve to determine the highest value reached. This value (which was rounded to the next integer) thus established the range, from 0%, for the highest effect in experimental data sets (groups or individuals) to be considered "within control". A JDE was then declared to have occurred when the *lower* 95% CI smoothed curve for data sets from exposure to MFG (groups or individuals) reached above this highest value.

Results

Establishing a just detectable effect (JDE)

Figure 2 presents the polynomial splines and 95% CI smoothed curves obtained for the classifications N, S, A, P and P1. These results are for the sham exposed groups which presented the largest deviation from 100% for N or from 0% for S, A, P and P1. Group

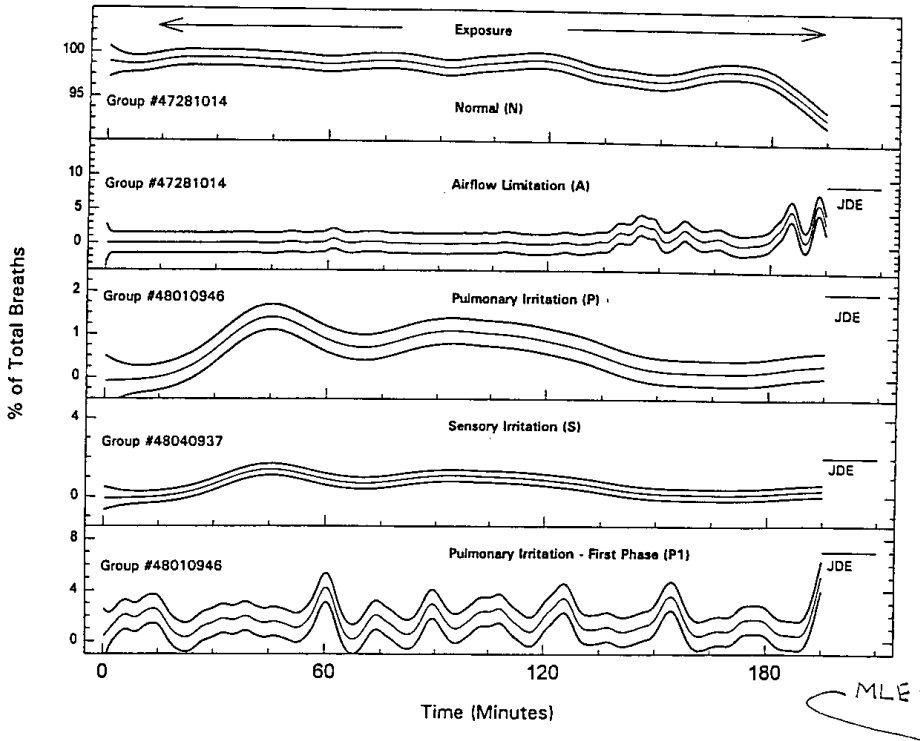


Fig. 2 Time-response analysis using the MLEGCV program for sham exposure groups results. The breath classifications smoothed polynomial spline curves (*thin center line*) with 95% CI, (*heavier lines*) were obtained using the stacked data sets for the group of animals. Data is presented as "% of Total Breaths" for a given classification for each collection period of 15 s. $n = 3120$ data points (four animals × 780 collection periods of 15 s each). A recurrence filter of 3 was used before processing the classification data with the MLEGCV program. The horizontal straight line at the right, identified as JDE, is rounded up to the next integer above the higher 95% CI line. If a value (lower 95% CI) for an exposure group is greater than the JDE value, an effect can be declared. Each curve for each breath classification was selected to represent the one group, out of the eight sham exposure groups, which had the largest deviation above 0%

#47281014 presented the largest deviation for A. This occurred towards the end of exposure and a corresponding decrease in the N classification occurred. Group #48010946 presented the largest deviation for both P1 and P while group #48040937 presented the largest deviation for S. The deviations from 0% were small, except for A, and the largest deviation for each abnormal category, above which a JDE would be declared for an exposed group are listed in Table 2. The results for the abnormal combinations, S + A, P + A, P + S and P + S + A are not presented. Their deviations above 0% were very small, less than 1% or simply 0%. For this reason, we entered the JDE values as 2% in Table 2, which is arbitrary, but is the same as for the S or P categories. Figures 3 and 4 present the polynomial spline and 95% CI smoothed curves obtained for the variables used to classify breaths. Again we selected the sham exposed group showing the largest variation for each variable. Group #48010946 showed the largest deviations for VT, TI, TE and TP while the largest deviations for V̇D and TB occurred with groups #47281014 and #48040937, respectively. The deviations from 100% (set as the control value) were very small. These deviations were not used to set a JDE, as with the breath classifications, but they illustrate the largest deviations for the variables used to classify breaths as S, A, P1 and P and these results will be used to estimate the intensity of the responses as discussed below.

The same approach was used to identify the largest deviations when inspecting the data for single animals with the results presented in Fig. 5 for the breath classifications, and in Fig. 6 for the variables V̇D, TP and TB and Fig. 7 for the variables VT, TI and TE. Different animals contributed in providing the largest variations, these variations being obviously larger than

Table 2 Just detectable effect (JDE) values for abnormal breath classifications from the results obtained for sham exposures[a]

Abnormal breath classification	Group data (% above which a JDE is declared)	Individual animal data (% above which a JDE is declared)
S	2	11
A	9	34[b], 20[c], 10[d]
P	2	13
PI	7	11
SA[e]	2	11
PS[e]	2	11
PA[e]	2	11
PSA[e]	2	11

[a] The percent value listed for each classification is the maximum increase above zero occurring at any time during a 3-h sham exposure period, determined from the *higher* 95% CI curve value obtained for a group or for an individual animal as shown in Figs 2 and 5. A JDE is declared if the *lower* 95% CI curve for an experimental group crosses above the value given for each classification

[b] 34% was the highest value obtained for an individual animal; the increase in A began to occur after 2 h of exposure

[c] 20% was the second highest value obtained for an individual animal; the increase in A began to occur after 2 h of exposure

[d] With the exception of the two animals mentioned above, all other animals ($n = 30$) exhibited A at levels of 10% or less

[e] All animals exhibited the combinations of breath classifications at very low percentages; therefore the values selected for S (2% or 11%), have been arbitrarily chosen as the values for these classifications

with group data. The JDE values for breath classifications when considering a single animal, are given in Table 2 from the data presented in Fig. 5. The results for the abnormal combinations, S + A, P + A, P + S and P + S + A are not presented, since the deviations above 0% were again very small and the JDE values entered in Table 2 were arbitrarily made equal to the S category.

Exposure to MFG

The results obtained were inspected as described above for the sham exposures, (i.e., for each group of animals and for each individual animal) at each exposure

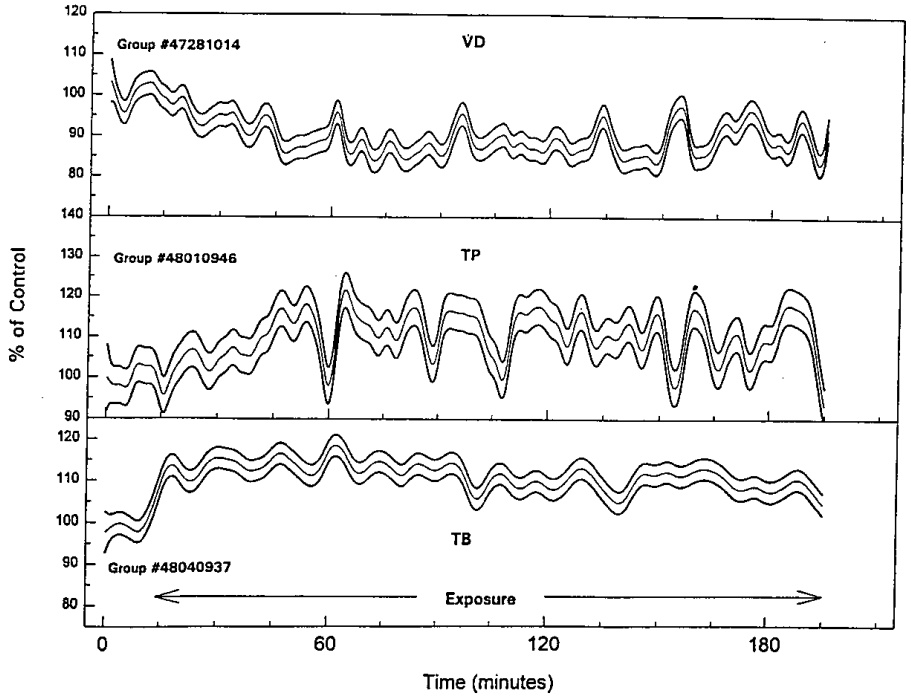


Fig. 3 Time-response analysis using the MLEGCV program for sham exposure groups results. The variables smoothed polynomial spline curves (*thin center line*) with 95% CI, (*heavier lines*) were obtained using the stacked data sets for the group. For each variable, the $\bar{X}$ value for the control period (first 15 min) was made equal to 100 and all values were plotted as a percentage of this value. $n = 3120$ data points (four animals × 780 collection periods of 15 s each). The $\bar{X}$ values were: $\dot{V}D = 3.889$ ml/s, TP = 0.023 s and TB = 0.025 s. The groups shown coincide with those shown in Fig. 2 for the corresponding breath classification (i.e., $\dot{V}D$ used for A, TP used for P and TB used for S)

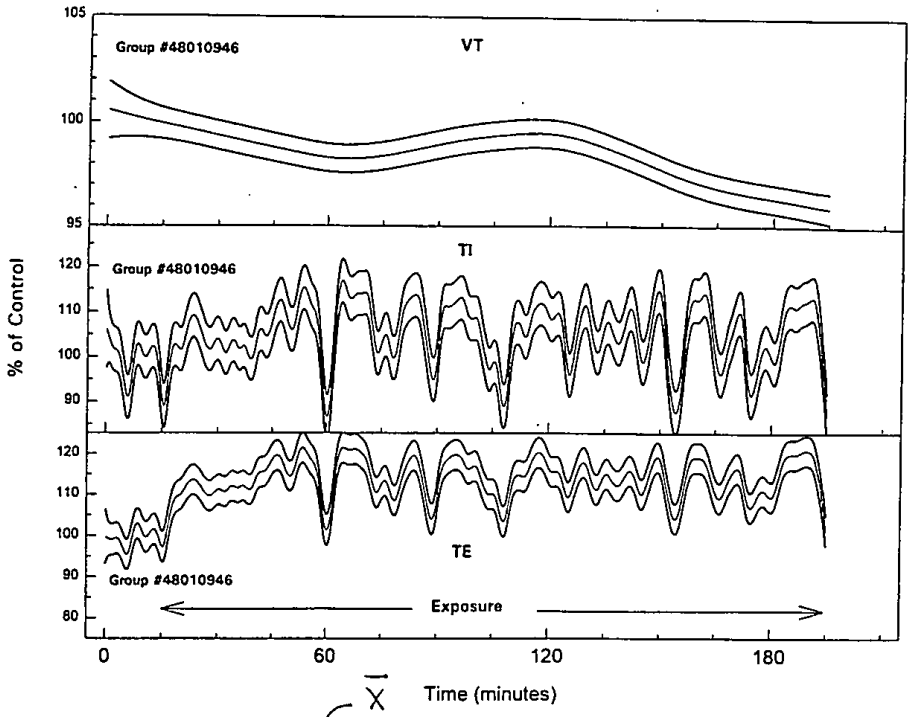


Fig. 4 Time-response analysis using the MLEGCV program for sham exposure groups results. The variables smoothed polynomial spline curves (*thin center line*) with 95% CI, (*heavier lines*) were obtained using the stacked data sets for the group of animals. For each variable, the $\bar{X}$ value for the control period was made equal to 100 and all values were plotted as a percentage of this value. $n = 3120$ data points (four animals × 780 collection periods of 15 s each). The values were: VT = 0.287 ml, TI = 0.075 s and TE = 0.121 s. The groups shown coincide with those shown in Fig. 2 for the corresponding breath classification (i.e., VT, TI, and TE used for P1)

concentration. The only significant group effects found (i.e., values above the JDE values listed in Table 2) were for the breath classifications S and P. No P1, A or combination effects were detected. The results are summarized in Fig. 8 with the addition of previous results for S and P obtained at higher concentrations: 70, 200 and 450 mg/m^3 (Boylstein et al. 1995b). For P, a group effect was observed at concentrations of 70 mg/m^3 and higher. There was also a significant group response at 0.2 mg/m^3, the level of response being 4%, just above the 2% value to declare a just detectable effect. However, as indicated in Fig. 8, we could not identify a response in individual animals in this group when inspecting their respective data sets, as opposed to the results at 70 mg/m^3 and higher. For S, the results were more complex, as also presented in Fig. 8. We found significant effects at low exposure concentrations, with great variability. This variability is further illustrated in Fig. 9. In this figure, we present both the results for the breath classification S and for the variable, TB, used to classify these breaths as S, which represents the intensity of the S reaction. Individual animal S reactions are shown in Fig. 9 for exposure concentrations from 2.08 to 70 mg/m^3, this last concentration being the lowest one at which 4/4 animals reacted. At the low concentrations, the reactions, all occurring at the beginning of exposure, were brief and not very intense. However, one animal clearly showed a reaction at 3.63 mg/m^3 which was as intense at the reaction obtained in one animal at 70 mg/m^3 and with a longer duration.

Discussion

Establishing a limit of detection or "just detectable effect"

In previous articles using these computer programs to recognize three types of possible effects along the respiratory tract and quantify the magnitude of each type

of effect, we placed the major emphasis on concentration-response analysis (Vijayaraghavan et al. 1993, 1994). From these concentration-response relationships, the potency of evaluated chemicals was expressed by selecting the mid-range of a possible maximum response. This is the classical bioassay approach, and when used, provided that a sufficient number of exposure concentrations are obtained, the low exposure concentrations results have very little influence on the potency estimate. However, when it is impossible to generate high exposure concentrations, the results at low exposure concentrations must be carefully scrutinized to decide whether or not an effect occurred. Thus, the limit of detection for a bioassay becomes crucial. Without knowing the limit of detection, it is impossible to affirm whether or not an effect was obtained at low exposure concentrations when using single animal data. Therefore, we used the approach described to establish a range of possible variation above 0% for sham exposed animals and declared a "just detectable effect" (JDE) to have occurred when a response just above this range occurred. This was accomplished for data sets of groups as well as for single animals. The JDE values obtained were quite low; thus this approach should be very useful to evaluate low exposure concentrations. Two exceptions to these low values were observed. This occurred for the A classification for 2/32 animals as listed in Table 2. The effect occurred suddenly after 2 h of sham exposure. There was no apparent reason for this sudden change. Possibly these two animals changed their position in their body plethysmograph in such a way as to impede normal breathing, but we cannot be certain.

Low versus high exposure concentrations

In order to evaluate the advantages of establishing a JDE we used a mixture, MFG, previously evaluated (Schaper and Detwiler 1991; Vijayaraghavan et al. 1993, 1994; Boylstein et al. 1995b). This mixture was selected for two reasons. First, the conclusion from previous results was that it can induce a variety of effects. S alone was observed at low concentrations, and S followed by P at moderate concentration and P alone or S alone followed by P at high concentrations. Also, an even more complex pattern of S, followed by A and P at high concentrations (Boylstein et al. 1995b). Thus, a much wider variety of effects could be induced than when using single chemicals. Second, of all airborne chemicals evaluated in our laboratory by the methods described here, the variability in the results obtained with this mixture in individual animals within an exposed group is the most pronounced. The results (Fig. 8) certainly confirm prior results and discussions on variability of data and that P predominates at high concentrations (and was previously properly quantified by Schaper and Detwiler, 1991) while S predominates

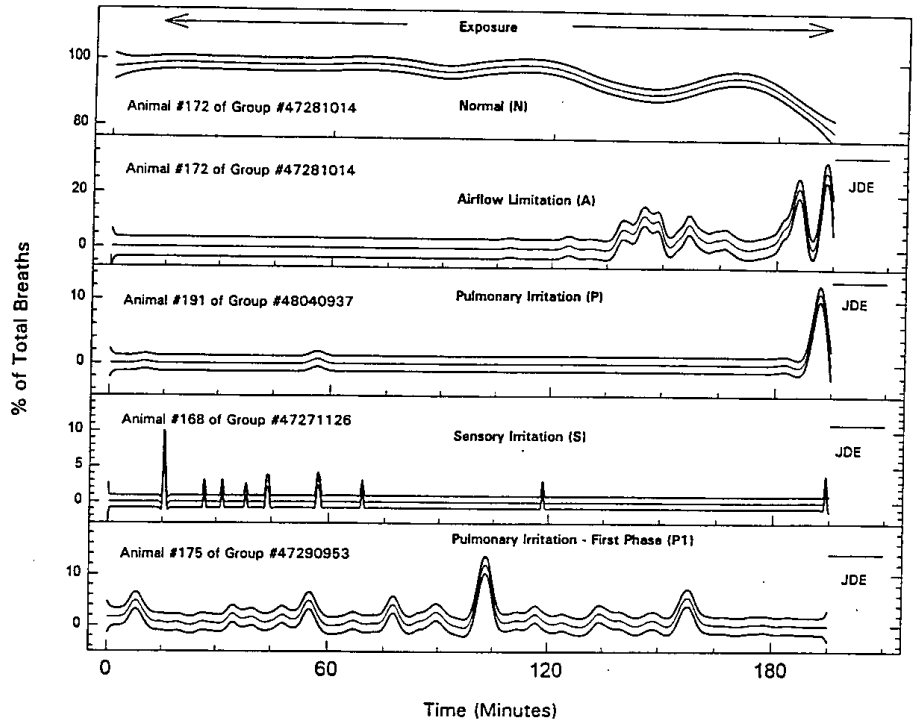
Fig. 5 Time-response analysis using the MLEGCV program for sham exposure single animal results. The classifications smoothed polynomial spline curves (*thin center line*) with 95% CI, (*heavier lines*) were obtained using data for an individual animal. Data is presented as "% of Total Breaths" for any given classification for each collection period of 15 s. $n = 780$ data points (780 collection periods of 15 s each for each animal). A recurrence filter of 3 was used before processing the classification data with the MLEGCV program. The horizontal straight line at the right, identified as JDE, is rounded up to the next integer above the higher 95% CI line. If a value (lower 95% CI) for an exposed animal is greater than the JDE value, an effect can be declared. Each curve for each breath classification was selected to represent the individual animal out of 32 (32 = four animals/group × eight groups) which had the highest values above 0%

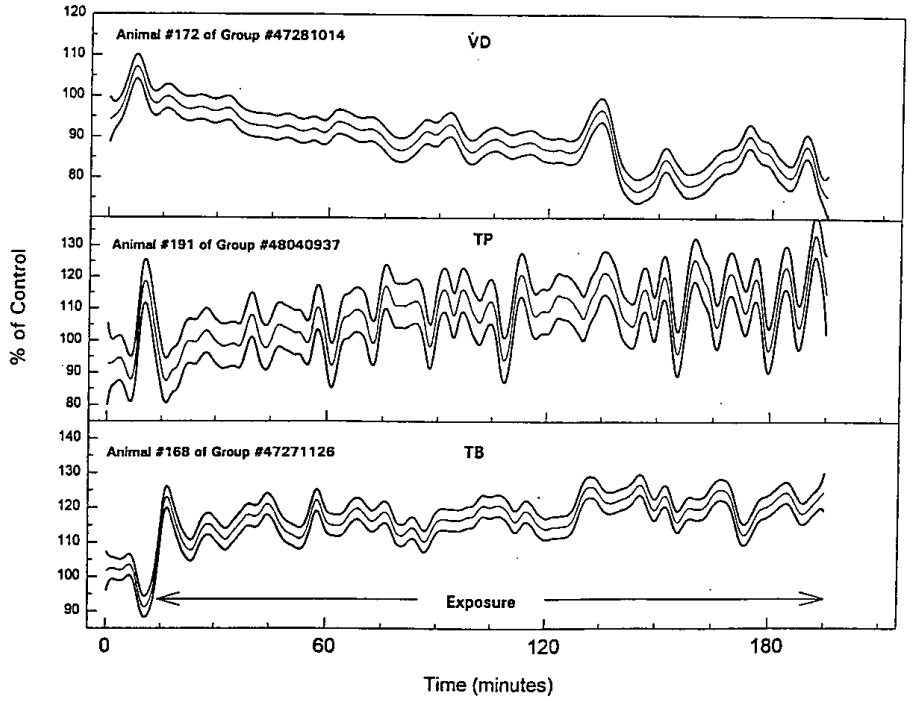
at low concentrations (but could not be firmly quantified until now). Thus establishing an occupational exposure limit such as a TLV based on the type of effect obtained at high concentrations becomes problematic, unless by chance, the safety factor applied happens to also prevent the different type of effect observed at low concentrations, if such an effect exists. A previous estimate for a TLV for MFG, based on its P effect, at high exposure concentrations was given as 7.5 mg/m^3 (Schaper and Detwiler 1991). This is close to the TLV of 5 mg/m^3 but it is above concentrations which induced an S effect of the low intensity and brief duration obtained at low exposure concentrations in mice (Fig. 9).

Variability of the data

As noted above, great variability in animal response was previously documented with this mixture. This is still the case for the low exposure concentrations results presented here, not for the P effect but certainly for the S effect. As shown in Fig. 8, an anomaly for the P effect is a group effect at 0.2 mg/m^3 while concentrations much above this, up to 55 mg/m^3, failed to induce this effect. It is worth noting that no individual animal effect was detected at 0.2 mg/m^3, only a group effect. For these reasons, despite all our efforts using statistical analysis of these data sets, it is likely that this effect was spurious rather than real. For the S effect, the variability is large between 1.25 and 55 mg/m^3. While we cannot explain such variability, we note that such has not

Fig. 6 Time-response analysis using the MLEGCV program for sham exposure, single animal results. The variables smoothed polynomial spline curves (*thin center line*) with 95% CI, (*heavier lines*) were obtained using data for an individual animal. For each variable, the $\bar{X}$ value for the control period (first 15 min) was made equal to 100 and all values were plotted as a percentage of this value. $n = 780$ data points (780 collection periods of 15 s each). The $\bar{X}$ values were: $\dot{V}D = 3.837$ ml/s, TP = 0.020 s and TB = 0.025 s. The animals shown coincide with those shown in Fig. 5 for the corresponding breath classification (i.e., $\dot{V}D$ used for A, TP used for P, and TB used for S)


Exposure
Animal #172 of Group #47281014
Normal (N)
Airflow Limitation (A)
Pulmonary Irritation (P)
Sensory Irritation (S)
Pulmonary Irritation - First Phase (P1)
JDE
% of Total Breaths
Time (Minutes)


Animal #172 of Group #47281014
VD
Animal #191 of Group #48040937
TP
Animal #168 of Group #47271126
TB
Exposure
% of Control
Time (minutes)

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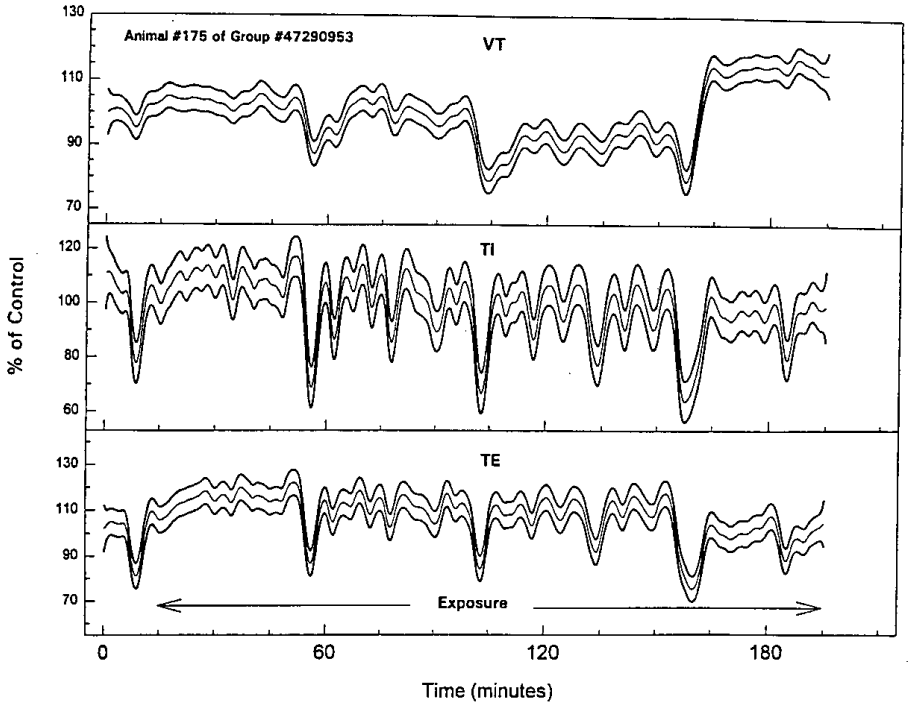

Fig. 7 Time-response analysis using the MLEGCV program for sham exposure, single animal results. The variables smoothed polynomial spline curves (*thin center line*) with 95% CI, (*heavier lines*) were obtained using data for an individual animal. For each variable, the $\bar{X}$ value for the control period (first 15 min) was made equal to 100 and all values were plotted as a percentage of this value. $n = 780$ data points (780 collection periods of 15 s each). The $\bar{X}$ values were: VT = 0.287 ml, TI = 0.075 s and TE = 0.121 s. The animals shown coincide with those shown in Fig. 5 for the corresponding breath classification (i.e., VT, TI and TE used for PI)

occurred with single chemicals previously (Vijayaraghavan et al. 1993, 1994) or currently under investigation at low exposure concentrations with the same protocol. Furthermore, the results cannot be explained as anomalous as in the case of the isolated P effect just noted above. Perhaps the only way to investigate this

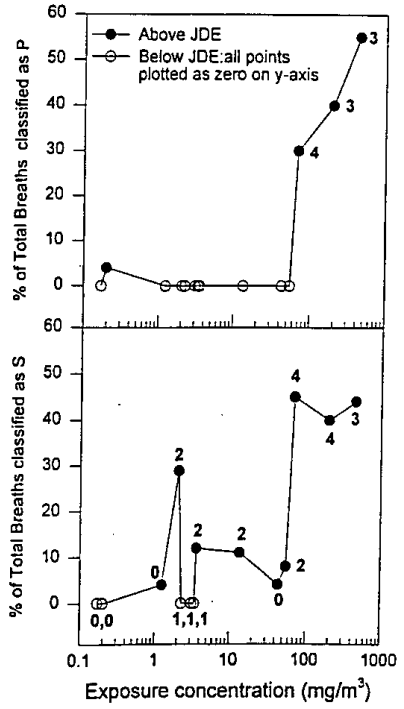

Fig. 8 Percent of total breaths classified as P or S at each exposure concentration. The value for each data point is the highest value reached during exposure, obtained from the smoothed polynomial spline curve (lower 95% CI curve) for the stacked data set of each exposure group. Each exposure group data point is identified as above or below the group JDE values listed in Table 2. The number of animals in each group of four animals reaching a value above the individual JDE values listed in Table 2 is indicated next to each point. For P, no individual animal reached a value higher than the JDE at exposure concentrations between 0.17 and 55 mg/m³. Data at 70, 200 and 450 mg/m³ are from Boylstein et al. (1995b). Note that at 450 mg/m³, the results are three animals for S as well as for P but 4/4 for either S or P response, (i.e. all animals responded but not in the same manner)

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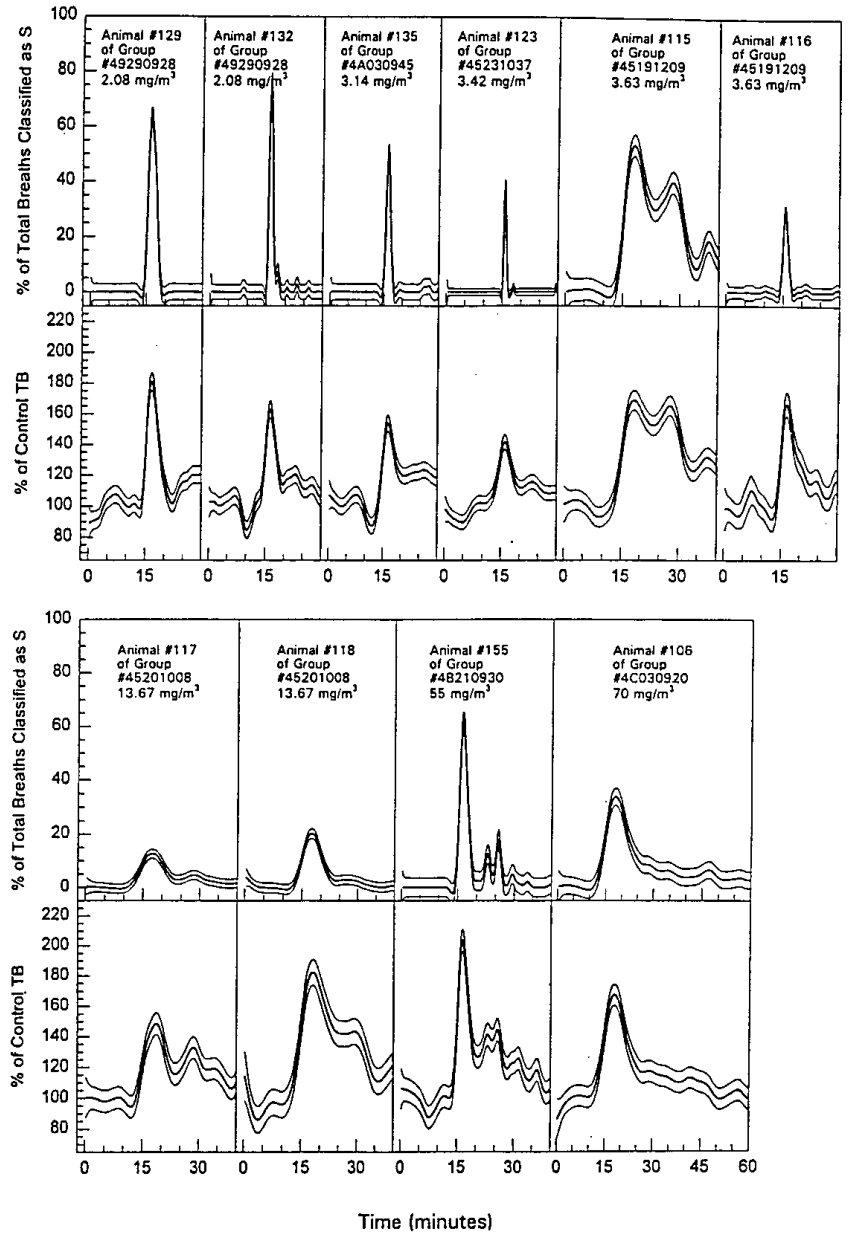

Fig. 9 Time-response analysis using the MLEGCV program for single animals exposed to concentrations ranging from 2 mg/m³ to 70 mg/m³ MFG. Data are shown for S classification and for the variable TB used for this classification in order to depict the intensity of the S reaction. The smoothed polynomial spline lower 95% CI curve reached above the JDE value for individual animals (11%) in all these examples and an effect is declared for each individual animal. A recurrence filter of 3 was used before processing the classification data with the MLEGCV program. Exposure was initiated at min 15

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phenomenon is to evaluate each component of this mixture. Nevertheless, the concentration range 1.25–13 mg/m^3 resulted in detectable S effects, either for the exposure group, single animals or both. For this reason, it is possible that some industrial workers may report sensory irritation in this exposure concentration range; the intensity of this effect being quite low in mice and of short duration (see Fig. 9), indicates that this would probably be an annoyance for humans rather than a stinging or burning sensory irritation level, as previously described with this animal model (Kane et al. 1978).

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Practical applications

It is often experimentally impossible to generate complex mixtures of airborne chemicals permitting the development of concentration-response relationships to estimate the potency of a mixture. Thus, if only low exposure concentrations can be generated, it is desirable to have a priori criteria to decide whether or not a particular effect has occurred. Since at low exposure concentrations it is likely that only a portion of the exposed animals will react, we need to define criteria for individual animals as well as for groups. With low JDE values obtained for this bioassay, low concentrations of complex mixtures can be investigated with confidence of reliably detecting possible effects. However, if only low exposure concentrations can be investigated, the results presented in Fig. 8 indicate that multiple exposures at low concentrations should be conducted. Otherwise, an isolated positive result as shown for the P effect in Fig. 8 cannot be adequately resolved.

This bioassay, as originally described by Vijayaraghavan et al. (1993) and further modified (Vijayaraghavan et al. 1994; Boylstein et al. 1995) uses a very simple inhalation exposure system. The series of computer programs for rule-based classification of the types of effect on the respiratory tract and to define the intensity of each effect can be easily implemented on 386 or 486 personal computers (386 or 486 PC); items commonly available in laboratories. The same programs have been recently used with guinea pigs (Stock et al. 1995). The same S, A and P effects were recognized as previously shown with the same chemicals used in mice (Vijayaraghavan et al. 1993, 1994). Furthermore, the A effect was also demonstrated as a result of aerosol challenges of guinea pigs previously sensitized by aerosol exposures to trimellitic anhydride. Thus, this bioassay can be implemented easily for two commonly used laboratory animal species. Further work with guinea pigs will be necessary to establish the JDE for each type of effect, as obtained in mice.

Acknowledgement The computer programs described in this article will be made available free of charge to interested investigators. The exposure system and body plethysmographs for mice or guinea pigs and aerosol generator used are available from Crown Glass Co., 990 Evergreen Drive, Somerville, NJ 08876, USA. We thank Miss M. Hirkulich for preparing the manuscript. We thank Dr. Schaper for supplying MFG used in this study as well as unpublished data on such mixtures. Supported under Grant No. RO1-ESO2747 from NIEHS.

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Prediction of an Occupational Exposure Limit for a Mixture on the Basis of Its Components: Application to Metalworking Fluids

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Using a previously developed mouse bioassay, a semisynthetic metalworking fluid (MWF "B") and its major components were evaluated. In mice MWF "B" and its components produced both sensory (S) and pulmonary (P) irritation. Using respiratory frequency (f) depression, concentration-response relationships were developed for each component as well as for MWF "B." From such relationships the concentration capable of evoking a 50% decrease in mean f was determined for each component and designated as $RD_{50}S$ if the decrease in f was due to sensory irritation, or $RD_{50}P$ if the decrease in f was due to pulmonary irritation. Based on $RD_{50}P$ values, the results indicated that the alkanolamides, potassium soap, sodium sulfonate, and triazine components were similar in irritation potency both to one another and to MWF "B." Through an examination of potency and fractional composition it was concluded that these five components largely contributed to the irritancy of MWF "B." From the $RD_{50}P$ values, occupational exposure limits that would protect workers from respiratory irritation were proposed for MWF "B" and each of its components. Using the approach of the American Conference of Governmental Industrial Hygienists for mixtures, an occupational exposure limit was calculated for MWF "B" employing the component data. The two limits for MWF "B" were similar to one another, suggesting that exposure limits for MWFs may be obtained through the evaluation of the fluids themselves or through evaluation of the components.

Keywords: alkanolamides, metalworking fluids, potassium soap, respiratory irritation, sodium sulfonate, triazine

It is estimated that over 10 million U.S. workers are exposed to machining or metalworking fluids (MWFs).[1] This figure includes employees of many industries who are involved in drilling, grinding, cutting, textile production, mist lubrication, and printing. Each application utilizes MWFs designed to best meet the particular needs of the job, resulting in a diverse array of available products, with further modifications often performed at the job site.

Three basic categories of MWFs were described by Key et al.[2] and were recently reviewed by Mackerer.[3] Petroleum-based mineral oils (60–100% naphthenic or paraffinic oil) known as straight oils or cutting oils are the oldest type of manufactured fluids. Straight oils may also contain small amounts of chlorine- or sulfur-based additives for high-pressure operations. Due to their excellent lubricating qualities these formulations are still popular. When water is added to oil-based fluids, soluble fluids are produced, containing 30–85% (v/v) oil. Emulsifiers, biocides, corrosion inhibitors, extreme pressure additives, antifoaming agents, dyes, and water conditioners

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may also be added to these MWFs. Often they are further diluted with water prior to use. Synthetic fluids are formulated without any oil, whereas semisynthetic fluids contain smaller amounts of oil, approximately 5–30% (v/v). Additives found in soluble fluids are also used in the synthetics and semisynthetics. The presence of water in soluble and synthetic MWFs improves their cooling effects, but also increases the potential for bacterial growth. For this reason biocides may be introduced in these MWFs to inhibit microbial and fungal growth. Biocides may be added by fluid manufacturers during formulation but are frequently added in the workplace while fluids are in use.

In 1950 Cruickshank and Squire[4] linked straight oil exposure with an increased incidence of skin cancer in English industrial workers. Since then there has been an increasing interest in defining the potential adverse health effects of exposure to MWFs. Protective apparel and improved engineering controls have helped to reduce cutaneous exposures in the workplace. However, there continues to be a concern with inhalation exposures to small aerosols (i.e., mass median aerodynamic diameter [MMAD] below 10 μm) formed during machining operations. These aerosols may be inhaled by workers, resulting in significant respiratory tract deposition and potential adverse health effects.[5-7]

Tolbert et al.[8] found that worker complaints have generally been associated with aerosol concentrations greater than 5 mg/m^3. Kennedy et al.[6] evaluated the forced expiratory volume during the first second of exhalation (FEV_1) in workers before and after their work shifts. For a decrease in FEV_1 of 5% or greater, the odds ratios were 4.4 among workers exposed to aerosols of straight oils, 5.8 for soluble fluids, and 6.9 for synthetic fluids. Cross-shift decreases were associated with inhalable aerosol levels above 0.20 mg/m^3. While FEV_1 decreased with higher exposure levels, these changes appeared reversible. Oxhoj et al.[9] compared respiratory symptoms of MWF-exposed workers to the general population, finding no spirometric differences between the groups, but noted a significant increase in cough and phlegm production in the exposed group. Although mild airway responses have been reported in MWF workers, it has not been possible to determine if they represent nonspecific irritant effects, or if there is a particular component(s) in the fluid that produces irritation at one or more levels of the respiratory tract. It is also possible that a specific component(s) may produce pulmonary sensitization, resulting in airway reactions on repeated exposures.

Few animal studies have evaluated the toxicological effects of MWFs, particularly focusing on acute respiratory reactions. Recently Schaper and Detwiler[10] assessed the sensory and pulmonary irritating properties of 10 different aerosolized MWFs using a mouse bioassay. Significant differences in biological potency were identified, with synthetics/semisynthetics and soluble fluids producing much more irritation than straight oils. As with the human data, it was not possible to determine from this animal study if the irritancy was a nonspecific effect of MWFs, or if it was related to a specific component(s) in the MWFs. However, the investigators felt that the irritancy was component-related and made some best guesses based on the chemical composition of each MWF.

The objectives of the present study were to assess the importance of components in defining the irritancy of MWFs and to demonstrate how component data might be used to predict an occupational exposure limit for an MWF. One of the 10 fluids used by Schaper and Detwiler[10] was MWF "B," a semisynthetic fluid. Its major components were evaluated here using the same mouse bioassay as employed previously. With the bioassay it would be possible to determine the relative irritation potency of the components and to suggest exposure limits for each component. Also, through examination of potency and fractional composition, the component(s) responsible for the acute respiratory effects seen by Schaper and Detwiler[10] could be identified. Finally, the component data could be used in the mixture formula of the American Confere of Governmental Industrial Hygienists (ACGIH)[11] to obtain occupational exposure limit for MWF "B." If successful this approach could be used to predict exposure limits for other mixtures besides MWFs.

Experimental Materials and Methods

Animals

Specific pathogen-free, male, Swiss-Webster mice were obtained from Hilltop Lab Animals (Scottdale, Pa.). They were housed in polypropylene cages, four per cage, with food (Purina Chow) and water provided *ad libitum*. The cages were kept in an animal room on a 12-hr dark/light cycle. A new group of four mice, weighing 24–28 g, was used for each experiment.

MWF "B" and Its Components

MWF "B" is a neat, semisynthetic fluid,[3] the sensory and pulmonary irritating properties of which have been previously described by Schaper and Detwiler.[10] The material safety data sheet (MSDS) for MWF "B" indicates that it is composed of water and seven other chemicals; its formulation is given in Table I. As noted in Table I, one component in MWF "B" is boramide. This is based on an expectation that when boric acid is added during production of MWF "B," it reacts with amines in the mixture to produce boramide. Therefore, boric acid as well as boramide were evaluated in this study. MWF "B" and its components (excluding water) v supplied to the University of Pittsburgh by the United Auto Wo.. ers and General Motors Corporation National Joint Committee on Health and Safety.

MWF "B" and its components were stored in their original sealed containers at room temperature until first opened. After this time they were kept in a cold room (approximately 5°C) until needed. Due to viscosity or chemical form (i.e., granular), some

TABLE I. Formulation of MWF "B"

Component	Fractional Concentration in MWF "B"	Fractional Concentration in MWF "B," *Excluding Water*
Alkanolamide #1 (tall oil acid diethanolamide)	0.05	0.109
Alkanolamide #2 (caprylic acid diethanolamide)	0.05	0.109
Boramide[A]	0.05	0.109
Petroleum oil	0.20	0.435
Sodium sulfonate	0.03	0.065
Triazine-type biocide (S-triazine, 1,3,5-2H,4H,6H-triethanol)	0.03	0.065
Potassium soap	0.05	0.109
Water	0.54	—

[A] Although boric acid is added to the formulation, it is expected that reac tion with amines yields boramide in the final product. Both boric acid and boramide were evaluated in this study.

components (e.g., boric acid) were diluted with deionized water to produce solutions for aerosolization.

Generation of Test Exposure

Using an infusion pump (Model 975, Harvard Apparatus, South Natick, Mass.), each component was fed into a Pitt No. 1 aerosol generator.[12] For several components a Pitt No. 4 generator[13] was also used to produce higher exposure concentrations. The rate of fluid delivery to the generator varied from approximately 0.015 to 3.3 mL/min. Dried compressed air was delivered to the generator (12 psi to Pitt No. 1, 20 psi to Pitt No. 4), producing a mist as the fluid met the airstream at the jet of the generator. The generator output was approximately 10 L/min of air, which was initially examined with a light source to assure that a mist was indeed being produced. Some volatilization of the aerosol droplets also occurred, thus resulting in the formation of vapors that were mixed with the aerosols. However, given the lower vapor pressures of the components in Table I, the extent of volatilization was not appreciable. Animals were exposed to components primarily in the aerosol phase.

Air samples were drawn at a rate of 2 L/min from the mouse exposure chamber onto glass fiber filters (Type A/E, 47-mm diameter, Gelman Sciences, Inc., Ann Arbor, Mich.). Gravimetric analysis was conducted for all components using a Mettler balance (Model AE240, Mettler Instrument Corp., Hightstown, N.J.). Exposure concentrations listed in "Results," below, reflect only nonvolatilized or solid components. Water and other volatilized components were excluded in these measurements.

At the RD_{50} concentration (or closest achievable concentration), particle sizing was performed using a Marple personal cascade impactor (Model 290, Andersen Samplers, Inc., Atlanta, Ga.). As shown in Table II, the MMAD of the aerosolized components ranged from 1–2 µm, and the geometric standard deviation (σ_g) was approximately 2. Thus, among this group of seven components, there was little variation in MMAD or σ_g values. Also presented in Table II are particle sizing results for MWF "B" obtained by Schaper and Detwiler.[10] The MMAD of 1.4 µm and σ_g of 1.5 for MWF "B" were similar to the data for the components.

Exposure Chamber and Measurements of Animal Respiration

The exposure chamber was made of glass and had a volume of 2.5 L. Each chamber had four body plethysmographs attached to it, as described by Barrow et al.[14] The four naive mice used for each exposure were positioned in a body plethysmograph with the head of each mouse extending into the interior of the chamber. A latex collar was used to create a seal around the neck of each mouse and to hold its head in place. A flowmeter was used to monitor the airflow through the chamber, with continuous ventilation at a rate of 20 L/min. A pressure transducer was attached to each body plethysmograph (Gaeltec 8T-2, Hackensack, N.J.) to measure plethysmographic pressure changes created by the mouse during inhalation and exhalation. Transducer output was directed into a Gould RS 3400 four-channel recorder, permitting the respiratory signals of each mouse to be continuously displayed. All signals (analog) were digitized at a rate of 200 samples/sec using a Metrabyte analog-to-digital converter (Model DAS-16, Taunton, Mass.) and stored on a Trillian Power Systems personal computer (Model II, 386 chip, Milpitas, Calif.).

The amplitude of the plethysmographic pressure changes, corresponding to thoracic displacement, was indicative of tidal volume (VT). Absolute calibration of VT was not performed, as only relative changes in VT were of interest here. Breathing frequencies (f) were measured for each of the four mice by counting the number of pressure waves per unit time. Individual and mean f values were calculated by the computer every 15 sec and displayed on the computer monitor. Following exposure, VT and f were plotted as a function of time for each animal. For each group of four exposed animals mean VT (± 1 standard deviation) and mean f (± 1 standard deviation) were also plotted as a function of time. A laser printer (Model LN03S-AA, Digital Equipment Corp., Maynard, Mass.) was used to generate these plots.

Each experiment consisted of a 20-min control period (in air alone), followed by a 180-min (3-hr) exposure to each component and a 20-min recovery period in air alone immediately following exposure. Mice were visually examined at 24–72 hrs postexposure. Some animals were also repositioned in the body plethysmographs at 24–72 hrs postexposure to evaluate potential changes in their respiratory patterns and f.

Recognition of Irritant Responses

As described by Alarie,[15,16] characteristic changes in respiratory patterns occur in mice exposed to sensory and pulmonary irritants. As sensory irritants stimulate trigeminal nerve endings in the nasal mucosa, the glottis closes and laryngeal resistance increases. This prolongation of the expiratory phase is seen as a braking pattern at the beginning of expiration. By prolonging expiration, f decreases. In contrast, pulmonary irritants stimulate vagal nerve endings, producing a pause between breaths in mice. Sensory and pulmonary irritants both evoke a decrease in f that is proportional to the exposure concentration.

Statistical Analysis

During 3-hr exposures mean f decreased until reaching a plateau in response that was then maintained until the end of exposure. This represented the time of maximum response, and here the percent decrease in f was determined with respect to control. A t-test ($p<0.05$) was used to test for significance. When significant decreases in f were found, they were examined as a function of the logarithm of exposure concentration. Least-squares regression analysis was then conducted to establish concentration-response relationships (i.e., testing that the slope of the line was significantly different from zero, $p<0.05$).[17] These relationships were used to calculate the exposure concentration resulting in a 50% decrease in mean f of exposed mice (i.e., RD_{50}).[16] At the time when this 50% decrease in mean f occurred, the respiratory patterns of mice were examined to identify the predominant type of irritation, sensory (S) or pulmonary (P). The RD_{50} was classified as $RD_{50}S$ if there was

TABLE II. RD_{50} and Particle Size at the RD_{50} for MWF "B" and Each of Its Components

Component	$RD_{50}P$[A] (mg/m³)	95% Confidence Interval	MMAD (µm)	σ_g
Alkanolamide #1	155	121–219	1.32	2.03
Alkanolamide #2	197	180–219	1.45	1.89
Boramide (boric acid)	NA	NA	1.93	1.99
Petroleum oil	3188	2310–5350	1.12	2.03
Sodium sulfonate	103	73–172	1.81	1.93
Triazine	137	90–206	1.41	2.33
Potassium soap	129	119–145	1.82	1.99
MWF "B"[B]	154	92–260	1.42	1.49

[A] $RD_{50}P$ signifies that at the time when a 50% decrease in mean f occurred, the predominant respiratory effect was pulmonary irritation (P).
[B] From Schaper and Detwiler,[10] $RD_{50}P$ also based on nonwater components

predominantly sensory irritation at this time, or $RD_{50}P$ if there was predominantly pulmonary irritation at this time.[18]

RESULTS

Changes in Breathing Patterns

Table III presents a summary of the changes in breathing patterns that were seen with each component of MWF "B."

Sensory Irritation

As noted in Table III, sensory irritation was evoked by all of the components in MWF "B." With aerosols of alkanolamide #2, boramide, petroleum oil, potassium soap, sodium sulfonate, and triazine, sensory irritation occurred immediately on exposure. Alkanolamide #1 and boric acid also produced this effect, but it occurred after approximately 30 min of exposure. Sensory irritation continued throughout the 3-hr exposures to the alkanolamides, boric acid, petroleum oil, triazine, and potassium soap. With boramide or sodium sulfonate, this effect faded after approximately 30 min of exposure. Boramide, boric acid, and sodium sulfonate produced mild sensory irritation in comparison with that from the other components.

Pulmonary Irritation

No pulmonary irritation occurred during the 3-hr exposures to boric acid or boramide aerosols. With the alkanolamides, petroleum oil, potassium soap, and triazine, pulmonary irritation generally occurred between the second and third hours of exposure. Thus, mixed patterns of sensory and pulmonary irritation were seen at this time. In contrast, sodium sulfonate aerosols produced pulmonary irritation within 30 min of exposure, and this was the predominant breathing pattern as sensory irritation faded.

Concentration-Response Relationships for Components of MWF "B"

Little change in VT occurred during exposures to the components in MWF "B." Thus, it was not possible to develop a concentration-response relationship using VT for any of the components. Also, the highest achievable concentration of boramide or boric acid was 300 mg/m^3, and exposure to these concentrations resulted in less than a 20% decrease in f. For this reason it was not possible to develop a concentration-response relationship using f for either of these components. As shown in Figure 1, exposure to the other components in MWF "B" resulted in concentration-dependent decreases in f. The concentration-response relations for MWF "B" was previously given by Schaper and Detwiler[10] and has been included in Figure 1 for comparison purposes.

Decreases in f were evoked on exposure to alkanolamide #1, alkanolamide #2, petroleum oil, and potassium soap, with a plateau in response generally occurring between the second and third hours of exposure. With triazine there were also decreases in f, but a plateau in response was seen somewhat earlier in the exposure (e.g., between the first and second hour of exposure). Interestingly, f increased for 10–15 min at the beginning of exposure to sodium sulfonate and then decreased as the exposure continued. At most concentrations of sodium sulfonate used in this study (90–200 mg/m^3) there was no plateau in response during exposure, and f continued to decrease during the third hour and often postexposure. Even when several exposures were extended to 4 hr, a plateau in response was not obtained. Immediately following exposure a moderate recovery (i.e., a partial return of f toward control levels) was seen with petroleum oil and potassium soap. Recovery was poor (i.e., little return of f toward control levels) immediately following exposure to the alkanolamides, sodium sulfonate, and triazine.

Postexposure Effects of Components of MWF "B"

Of all the components, the triazine-type biocide produced the most severe adverse effects on the animals after exposure. Following exposure to triazine, animals lost weight, and many appeared to be breathing through their mouths and were gasping. They also made audible clicking or chirping sounds. Both sens and pulmonary irritation patterns were seen at this time. Triaz.. was the only component that resulted in deaths within 24–72 hrs postexposure.

DISCUSSION

The concentration-response relationships of the alkanolamides, potassium soap, sodium sulfonate, and triazine were clustered together and located far to the left of that for the petroleum oil component. Also, the slopes of the concentration-response relationships for the alkanolamides, potassium soap, sodium sulfonate, and triazine were similar to one another and were much steeper than that for the petroleum oil. Interestingly, the concentration-response relationship for MWF "B" was located among those for the alkanolamides, potassium soap, sodium sulfonate, and triazine, yet the slope of the curve for MWF "B" most closely matched that of its petroleum oil component.

From these relationsl the concentration capable of producing a 50% decrease in mean f was determined for

TABLE III. Acute Respiratory Responses to MWF "B" and Each of Its Components

Component	Sensory Irritation	Pulmonary Irritation	Plateau in Response	Recovery Immediately Postexposure
Alkanolamide #1	yes, 0.5–3 hr	yes, 2–3 hr	yes, 2–3 hr	poor
Alkanolamide #2	yes, 0–3 hr	yes, 2–3 hr	yes, 2–3 hr	poor
Boramide	yes, but fades after 0.5 hr	no	yes, 0–1 hr	yes
Boric acid	yes, 0.5–3 hr	no	yes, 2–3 hr	yes
Petroleum oil	yes, 0–3 hr	yes, 2–3 hr	yes, 2–3 hr	moderate
Potassium soap	yes, 0–3 hr	yes, 2–3 hr	yes, 2–3 hr	moderate
Sodium sulfonate	yes, but fades after 0.5 hr	yes, 0.5–3 hr	generally, no	poor
Triazine	yes, 0–3 hr	yes, 2–3 hr	yes, 1–2 hr	poor, death within 24–72 hr
MWF "B" [A]	yes, 0–3 hr	yes, 2–3 hr	yes, 2–3 hr	poor

[A] From Schaper and Detwiler[10]

each component (i.e., RD_{50} values). At the time when a 50% response occurred, the predominant type of respiratory effect was pulmonary irritation. Thus, Table II lists $RD_{50}P$ values to reflect this finding. On the basis of $RD_{50}P$ values, the relative potency of the components of an MWF "B" was: alkanolamide 1 ≈ alkanolamide 2 ≈ potassium soap ≈ sodium sulfonate ≈ triazine ≫ petroleum oil. All had $RD_{50}P$ values between 100–200 mg/m³ except petroleum oil, the $RD_{50}P$ value of which was 3188 mg/m³, indicating that it was much less potent than any of the other components.

Based on similarities in potency (i.e., equivalent $RD_{50}P$ values) and fractional composition (i.e., 3–5% of each in the fluid), it appeared that the two alkanolamides, potassium soap, and triazine all contributed to the irritancy of MWF "B." The petroleum oil had a minor contribution in comparison to these other components. Likewise, boramide and boric acid were mild sensory irritants and contributed little to the irritancy of MWF "B."

Alarie[19] and Alarie and Luo[20] have shown that the mouse bioassay may be used to predict human responses. At the $RD_{50}S$ humans would experience intolerable burning of the eyes, nose, and throat, but at 0.03 × $RD_{50}S$ (or 1/30 × $RD_{50}S$), sensory irritation may be prevented in humans. Schaper[21] recently developed a database of 295 airborne materials, the sensory irritating properties of which were evaluated using the mouse bioassay. She also examined the relationship between the ACGIH[11] threshold limit value (TLV®) and 0.03 × $RD_{50}S$. As previously shown by Alarie[19] and Alarie and Luo[20] for 26 and 40 chemicals, she found a strong correlation between TLVs and 0.03 × $RD_{50}S$ values for a larger number of chemicals (i.e., 89). For pulmonary irritants no formal relationship between the TLV and $RD_{50}P$ has yet been established. Weyel et al.[22] and Weyel and Schaffer[23] recommended that the $RD_{50}P$ be divided by 60 to obtain an exposure concentration that will protect workers from pulmonary irritation. Thus, the use of 1/60 × $RD_{50}P$ serves as a best guess for pulmonary irritants at the present time.

In Table IV occupational exposure limits are proposed for components of MWF "B" based on pulmonary irritation (1/60 × $RD_{50}P$). Because an exposure limit based on pulmonary irritation is lower than one based on sensory irritation (1/60 × $RD_{50}P$ versus 1/30 × $RD_{50}S$), it will protect workers from both types of respiratory irritation. An exposure limit of approximately 2 mg/m³ is suggested for the alkanolamides, potassium soap, sodium sulfonate, and triazine. At this time there are no TLVs or Occupational Safety and Health Administration permissible exposure limits (PELs)[24] for any of these chemicals. Thus, 2 mg/m³ should be a good starting point for them, but this proposed limit may need to be lowered as additional data (e.g., toxicological or epidemiological) become available, possibly suggesting other health effects (e.g., reproductive/developmental toxicity, cancer).

TABLE IV. Proposed Exposure Limits for MWF "B" and Each of Its Components

Component	$RD_{50}P$ (mg/m³)	1/60 × $RD_{50}P$ (mg/m³)
Alkanolamide #1	155	2.6
Alkanolamide #2	197	3.3
Petroleum oil	3188	53.1
Sodium sulfonate	103	1.7
Triazine	137	2.3
Potassium soap	129	2.2
MWF "B"[A]	154	2.6

[A] From Schaper and Detwiler[10]

For the petroleum oil component, an occupational exposure limit of 53 mg/m³ is proposed to prevent respiratory irritation. Schaper and Detwiler[10] suggested an exposure limit of approximately 1000 mg/m³ for a sulfonated straight oil (MWF "F"). Because these oils were not chemically identical, differences in potency and exposure limits were not surprising. Nevertheless, the current TLV or PEL of 5 mg/m³ for an oil mist should be acceptable for MWF "F" as well as the petroleum oil component of MWF "B." However, 5 mg/m³ may be too high for the mixture (MWF "B") for which Schaper and Detwiler[10] suggested an occupational exposure limit of 2.6 mg/m³. Using component data from the present study, this value was re-evaluated.

ACGIH[11] provides guidance regarding TLVs for mixtures, noting that when there are two or more substances in a mixture that produce the same type of toxic effect, their combined effects must be given primary consideration. If there are no data to suggest otherwise, their effects may be considered additive. Equation 1 is to be used when the mixture is a liquid and the atmospheric composition is assumed to be similar to that of the original material.

$$\frac{1}{fc_a/TLV_a + fc_b/TLV_b + fc_c/TLV_c + \ldots fc_n/TLV_n} = \text{TLV of the mixture}$$

It is appropriate to apply Equation 1 to the data generated in this study because (1) MWF "B" is a liquid mixture with an atmospheric composition that is expected to reflect its major components, and (2) the components in MWF "B" produce the same type of toxic action. That is, the alkanolamides, petroleum oil, sodium sulfonate, triazine, and potassium soap all produced respiratory irritation, predominantly pulmonary irritation when there was a 50% decrease in mean *f*. This indicated that the components in MWF "B" were capable of acting on trigeminal nerve endings in the upper respiratory tract (i.e., nose) as well as vagal-type nerve

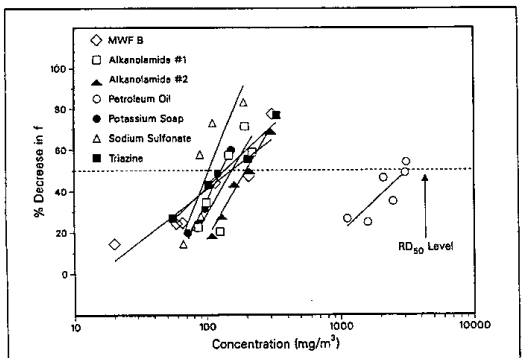

FIGURE 1. Concentration-response relationship for MWF "B" and each of its components, except boramide and boric acid. It was not possible to develop a concentration-response relationship for boramide or boric acid. Each point shown here represents the mean response of four mice (i.e., percent decrease in mean *f*) during 3-hr exposures. Linear least squares regression analysis revealed that the slope of each line was significantly different from zero ($p < 0.05$). The concentration-response relationship for MWF "B" was previously obtained by Schaper and Detwiler.[10]

endings in the lower respiratory tract (i.e., alveolar region). Thus, there are good mechanistic reasons to anticipate additivity of the effects of these components.

With MWF "B," the fractional concentration of each component (fc) was provided on its MSDS. However, when sampling for MWF "B" from the mouse exposure chamber or in the workplace, water will be excluded. Therefore, fc of each component was calculated without water, as given in Table I. In Table IV there are estimates of occupational exposure limits (i.e., possible TLVs to prevent pulmonary irritation) for each component in MWF "B" except boramide. These data were used in Equation 1, yielding an exposure limit of 5.0 mg/m^3 for MWF "B;" the contribution of boramide was assumed to be negligible. This value (i.e., 5 mg/m^3) was slightly higher than the previously proposed limit of 2.6 mg/m^3 for MWF "B."[10] Within the limitations of the mouse bioassay these values are not different from one another. Most importantly, this study has demonstrated that an exposure limit for MWF "B" could be predicted from evaluation of the fluid itself or from evaluation of its individual components. The approach used in the present study should be applied to other MWFs and their components to further validate this conclusion.

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Correction

In November 1995 the *AIHA Journal* published "Development of a New Qualitative Test for Fit Testing Respirators" (56:1068–1073). In this article the authors referenced draft American National Standards Institute (ANSI) standard Z88.10, which attempts to establish acceptable respirator fit testing methods. John Hale, chairman of the Z88.10 committee, and James Johnson, secretary of the ANSI Z88 respirator standards, have both indicated that referencing this draft at this time was inappropriate, as the standard remains in a dynamic state.

Although other published peer-reviewed articles, including those appearing in this journal, have referenced draft ANSI standards, we are concerned that allowing the citation of this standard may have given the impression that what constitutes a valid respirator fit test has been adequately discussed and agreed to by members of this committee. It is our understanding from Mr. Hale and Dr. Johnson that this item is still very much in the discussion stages. We wish to point out to our readers that the article should not have cited this draft standard. However, the authors did cite other studies in the article that describe validation procedures for respirator fit tests.—*Howard J. Cohen, Ph.D., CIH; Editor in chief*

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ORIGINAL INVESTIGATION

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Respiratory effects of a synthetic metalworking fluid and its components

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Abstract A synthetic metalworking fluid, MWF "A", and its major components were evaluated using a previously developed mouse bioassay. This fluid and its components evoked sensory (S) and pulmonary (P) irritation in mice. For MWF "A" and each of its components, a concentration-response relationship was developed on the basis of respiratory frequency (f_R) responses. From such relationships, the concentration capable of evoking a 50% decrease in mean f_R was determined for MWF "A" and each component (RD_{50}). $RD_{50}S$ or $RD_{50}P$ was used to distinguish decreases in f_R that were due to sensory irritation (S) from those due to pulmonary irritation (P). From $RD_{50}P$ values, it was concluded that the fatty acid alkanolamide condensates, tolutriazole, and triazine-type biocide components were similar in potency to one another and similar in potency to MWF "A". By examining potency and fractional composition, it was concluded that the fatty acid alkanolamide condensates and the triazine-type biocide largely contributed to the irritancy of MWF "A". From $RD_{50}P$ values, occupational exposure limits were proposed for MWF "A" and each of its components. The current Threshold Limit Value of 10 mg/m^3 established by the American Conference of Governmental Industrial Hygienists for "particulates not otherwise classified" (PNOC) would be inadequate to protect workers from the irritating properties of MWF "A" and most of its components.

Key words Respiratory effects · Metalworking fluid · Mouse bioassay

Introduction

Metalworking fluids (MWFs) are widely used in industrial operations such as cutting, drilling, and grinding. In the older scientific literature, these fluids were described as "cutting oils" and were generally petroleum-based mineral oils (Mackerer 1989). Because of their good lubricating qualities, "cutting oils" are still found in the workplace. However, other types of MWFs have been introduced for use in industrial operations. One newer class of MWFs are the *synthetics*, which are complex chemical mixtures, formulated with components such as acids, alcohols, amines, and sulfonates. Unlike the traditional "cutting oils", they do not include any oil. The *"neat" synthetics*, when manufactured, often contain large proportions of water (e.g., 50–60%) and are further diluted with water (e.g., 2–20%) in the workplace. The presence of water enhances their cooling properties, but it also increases the potential for growth of microorganisms. Triazine-type biocides may be added to synthetic fluids to inhibit such growth.

There are few studies (epidemiological or toxicological) that have evaluated the potential human health effects of newer types of MWFs such as the synthetics. Given the high pressures and temperatures under which these MWFs are used in the workplace, aerosols may be formed whose aerodynamic size is well below 10 μm (Ayer 1964; Kennedy et al. 1989; Chan et al. 1990). Inhalation exposures of workers may then occur. MWF aerosols may be deposited and retained throughout the respiratory tract, thereby eliciting a variety of respiratory reactions. Considering the large number of workers exposed to MWFs and the potential for repeated exposures, it is important to have information regarding the pulmonary toxicity of newer types of MWFs as well as the components used in formulating these fluids.

In a recent study, the acute respiratory effects of ten different aerosolized MWFs were evaluated using a mouse bioassay (Schaper and Detwiler 1991). One *synthetic* fluid, MWF "A", was included in the study. It produced both sensory and pulmonary irritation in mice and was the most potent of the ten fluids evaluated in the study. However, it was not possible to determine which component(s) in MWF "A" was (were) responsible for its irritancy. It was speculated that the amines in MWF "A" contributed to this effect (Schaper and Detwiler 1991).

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For the present study, the objectives were to evaluate the irritancy of components in MWF "A" and to compare component data with data previously obtained for the fluid itself (Schaper and Detwiler 1991). The major components of this *synthetic* fluid were evaluated using the same mouse bioassay as employed by Schaper and Detwiler (1991). Using the bioassay, relative potency of the components could be assessed and exposure limits could be suggested for each component. Also, through examination of potency and fractional composition, the component(s) responsible for the acute respiratory effects of MWF "A" reported by Schaper and Detwiler (1991) could be identified. Finally, the data for MWF "A" and its components could be used to provide some guidance regarding occupational exposure limits for *synthetic* MWFs.

Materials and methods

Animals

Specific pathogen-free, male, Swiss-Webster mice were obtained from Hilltop Lab Animals (Scottdale, Pa., USA). They were housed in polypropylene cages, four per cage, with food (Laboratory Rodent Diet 5001, PMI Feeds, Richmond, Ind., USA) and water ad libitum. The cages were kept in an animal room which was held at a temperature of 70–72 ° F, at 40–50% relative humidity, and on a 12-h dark/light cycle. A new group of four mice, each weighing 24–28 g, was used for each experiment.

Metalworking fluid (MWF) "A" and its components

MWF "A" may be described as a neat synthetic fluid (i.e., a fluid not yet introduced in the workplace). As listed in Table 1, there are 12 components in MWF "A". The major component of MWF "A" is water (>60% v/v); however, it was not evaluated in this study. MWF "A" and the other 11 chemical components were supplied to the University of Pittsburgh by the United Auto Workers (UAW) and General Motors Corporation (GM) National Joint Committee on Health and Safety. MWF "A" and its components were stored at room temperature until first opened. Thereafter, they were kept in a cold room (approximately 5 °C).

Generation and characterization of test exposure

Using an infusion pump (Model 975, Harvard Apparatus, South Natick, Mass., USA), each component was fed into a Pitt No. 1 aerosol generator (Wong and Alarie 1982). For several components, a Pitt No. 4 generator (Rosato et al. 1988) was also used in order to produce higher exposure concentrations. The rate of fluid delivery to the generator varied from approximately 0.015–3.3 ml/min. Dried, compressed air was also delivered to the generator (12–20 psi). The output of the generators was passed in front of a high-intensity lamp to observe if a mist was being produced. Generator output was approximately 10 l/min, which was directed into the mouse exposure chamber (described below).

With the exception of 2-amino-2-methyl-1-propanol (AMP), the other components of MWF "A" were nebulized and mist concentration was determined gravimetrically (Mettler balance, Model AE240, Mettler Instrument Corp., Hightstown, N.J., USA). Air samples were drawn at a rate of 2 l/min from the mouse exposure chamber onto glass fiber filters (Type A/E, 47 mm diameter, Gelman Sciences, Ann Arbor, Mich., USA). For AMP, air samples were drawn from the mouse exposure chamber into a Miran Infrared Analyzer (Model IA, Foxboro Co., East Bridgewater, Mass., USA) and absorbance was read at 9.5 nm.

Table 1 Information from material safety data sheet (MSDS) for MWF "A"

Component (abbreviation)	Fractional concentration in MWF "A"
2-Amino-2-methyl-1-propanol (AMP)	0.01–0.1
Diisopropanolamine (DIA)	0.01–0.1
Fatty acid alkanolamide condensates (FAC)	0.01–0.1
Isononanoic acid (INA)	0.01–0.1
Monoisopropanolamine (MIA)	0.01–0.1
Phosphonate sequestrant (PPS)	<0.01
Siloxane (SIL)[a]	<0.01
Sodium pyrithione (SPY)[b] (2-pyridinethiol-1-oxide, sodium salt)	<0.01
Tolutriazole (TOL)	<0.01
Triazine-type biocide (TRI)[b] (hexahydro-1,3,5 tris (2-hydroxyethyl)-*S*-triazine)	0.01–0.1
Triisopropanolamine (TIA)	0.01–0.1
Water	>0.60

[a] This component is actually a mixture of dimethyl siloxanes and oxiranes and is used as a defoamer in MWF "A"
[b] This component is sometimes called a preservative

It is important to note that gravimetric analyses only reflected the capture of chemicals in the solid state or those with a lower vapor pressure. In contrast, infrared analyses permitted detection of chemicals with a higher vapor pressure, but specifically those absorbing at a particular wavelength (i.e., 9.5 nm). Irrespective of the analytical method used for components in this study, water vapor was not included in any determination of exposure concentration. This was similar to the approach used in the evaluation of MWF "A" itself (Schaper and Detwiler 1991). Thus, exposure concentrations obtained with this synthetic fluid could be compared to those obtained with its components.

At the RD_{50} concentration (or closest achievable concentration), particle sizing was performed using a Marple personal cascade impactor (Model 290, Andersen Samplers, Atlanta, Ga., USA). The mass median aerodynamic diameter (MMAD) of aerosolized components ranged from 1 to 2 μm and the geometric standard deviation (σ_g) was approximately 2. These data were consistent with previous results of particle size analysis for MWF "A" and other MWFs which were reported by Schaper and Detwiler (1991).

Exposure chamber and measurements of animal respiration

The exposure chamber was 2.5 l in volume and was made of glass (Barrow et al. 1977). It was continuously ventilated at a rate of 20 l/min. Four body plethysmographs were attached to the exposure chamber and a mouse was positioned in each plethysmograph such that its head extended into the interior of the chamber. A latex collar served as a seal around the neck of each mouse and held its head in place. Pressure transducers were attached to the plethysmographs (Gaeltec 8T-2, Hackensack, N.J., USA), permitting the measurement of pressure changes created by mice during inhalation and exhalation. Transducer output was directed into a four-channel recorder (Gould, Model RS 3400, Cleveland, Ohio, USA) to display the respiratory signals of each mouse. Analog signals were digitized at a rate of 200 samples/s using an analog to digital converter (Metrabyte, Model DAS-16, Taunton, Mass., USA) and stored on a personal computer (Trillian Power Systems, Model II, 386 chip, Milpitas, Calif., USA).

The amplitude of the plethysmographic pressure changes, corresponding to thoracic displacement, was indicative of tidal volume (V_T). In this study, absolute values for V_T were not obtained and thus only relative changes in V_T are reported here. The respiratory frequency (f_R) of each mouse was determined by counting the number of pressure waves per unit time. Individual and mean f_R values were calculated by the computer every 15 s and displayed on the computer monitor. Following exposure, V_T and f_R were plotted as a function of time for each animal. For a group of four animals, mean V_T ($\pm$ 1 SD) and mean f_R ($\pm$ 1 SD) were also plotted as a function of time. A laser printer (Model LN03S-AA, Digital Equipment Corporation, Maynard, Mass., USA) was used to generate these plots.

Length of exposure

Each experiment consisted of a 20-min control period (in air alone). Animals were then exposed to room air (i.e., sham-exposed), or to MWF "A", or to one of its components for a period of 180 min. A 20-min recovery (in air alone) was given immediately following exposure. Animals were then returned to their cages and kept for approximately 1 week, during which time any mortalities were recorded.

Recognition of irritant responses

Through evaluation of the individual breathing patterns of mice, it is possible to determine the level(s) of the respiratory tract at which a chemical is acting.

Sensory irritation

Exposure to sensory irritants (such as acrolein and formaldehyde) results in a stimulation of trigeminal nerve endings in the oral-nasal mucosa and there is closure of the glottis with an increase in laryngeal resistance. The first stage of expiration is prolonged because of this closure (i.e., "braking" occurs) (Alarie 1966, 1973; Vijayaraghavan et al. 1993, 1994). By prolonging expiration, f_R decreases. This effect is concentration-dependent.

Pulmonary irritation

In contrast, exposure to pulmonary irritants (such as nitrogen dioxide and propranolol) results in stimulation of vagal nerve endings within the lower respiratory tract. Such chemicals act directly and/or indirectly via pulmonary hemorrhage, congestion and edema (Schaper et al. 1989; Schaper and Brost 1991; Vijayaraghavan et al. 1993, 1994). The action of pulmonary irritants on vagal nerve endings produces a "pause" between breaths in mice which then results in a decrease in f_R (Alarie 1973; Vijayaraghavan et al. 1993, 1994). This effect is also concentration-dependent.

Statistical analysis

Using mean responses, the maximum decrease in f_R that occurred during the 180 min exposure was determined with respect to control. A paired t-test ($p < 0.05$) was used to test for significance. When significant decreases in f_R were found, they were examined as a function of the logarithm of exposure concentration. Least-squares regression analysis was then conducted to develop concentration-response relationships (i.e., testing that the slope of the line was significantly different from zero, $p < 0.05$) (Armitage 1977). These relationships were used to calculate the exposure concentration resulting in a 50% decrease in mean f_R of exposed mice (i.e., RD_{50}) (Alarie 1973). At the time when this 50% decrease in mean f_R occurred, the individual respiratory patterns of mice were examined to identify the predominant type of irritation, sensory (S) or pulmonary (P). The RD_{50} was classified as $RD_{50}S$ if there was predominantly sensory irritation at this time or $RD_{50}P$ if there was predominantly pulmonary irritation at this time (Krystofiak and Schaper 1995; Schaper and Detwiler-Okabayashi 1995).

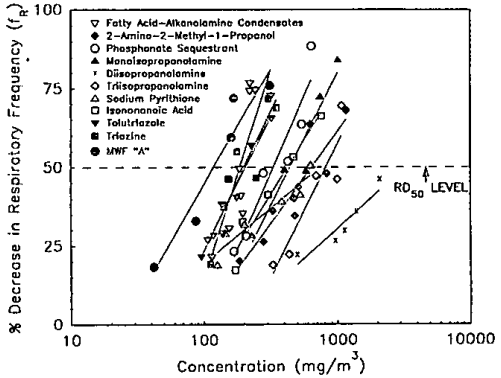

Fig. 1 Concentration-response relationship for MWF "A" and its components, except SIL. It was not possible to develop a concentration-response relationship for this defoaming agent. The remaining components and MWF "A" evoked a decrease in f_R which generally reached a maximum in the third hour of exposure. Each point shown here represents the mean response of four mice (i.e.,% decrease in mean f_R) taken at this time. Linear least squares regression analysis revealed that the slope of each line was significantly different from zero ($p < 0.05$). The concentration-response relationship for MWF "A" was previously obtained by Schaper and Detwiler (1991)

Results

Respiratory effects of sham exposure

Mice exposed to room air for a period of 220 min exhibited no evidence of sensory or pulmonary irritation. There were no changes in f_R or V_T at any time during the sham exposure.

Concentration-response relationships for MWF "A" and its components

Figure 1 presents the concentration-response relationship for MWF "A" and for each of its components, except SIL. It was not possible to generate exposure concentrations above 100 mg/m^3 with this defoaming agent, and little response was seen during exposures that were conducted (i.e., under 15% decrease in mean f_R due to sensory irritation). For the other ten components as well as MWF "A" itself, the concentration range used for exposures was 40–2000 mg/m^3. Lower concentrations were used for exposures to MWF "A", FAC, TOL, and TRI (e.g., 40–400 mg/m^3) while much higher concentrations were used for DIA (e.g., 800–2000 mg/m^3). For the remaining components, AMP, INA, MIA, PPS, SPY, and TIA, the

Table 2 $RD_{50}P$ values and proposed occupational exposure limits for MWF "A" and each of its components

Component	$RD_{50}P$ (95% confidence interval) (mg/m³)		1/60 × $RD_{50}P$ (mg/m³)
MWF "A"[a]	120	(82–171)	2.0
FAC	190	(161–219)	3.2
TRI	190	(153–239)	3.2
TOL	205	(187–233)	3.4
PPS	330	(256–426)	5.5
INA	420	(337–589)	7.0
MIA	440	(277–593)	7.3
AMP	640	(508–915)	10.7
SPY	740	(502–1775)	12.3
TIA	815	(570–1069)	13.6
DIA	3200	(1940–32832)	53.3
SIL	–		–

[a] From Schaper and Detwiler (1991)

exposure concentrations were mid-range, falling between these upper and lower bounds.

Based upon the relationships in Fig. 1, the concentration producing a 50% decrease in mean f_R (i.e., RD_{50}) was obtained for MWF "A" and each of its components. These values and the associated 95% confidence intervals are given in Table 2. They are reported as $RD_{50}P$ values because there was evidence of pulmonary irritation at the time when maximum decreases in f_R occurred during exposure. In Table 2, the $RD_{50}P$ values have been ordered from smallest (i.e., 120 mg/m³ for MWF "A") to largest (i.e., 3200 mg/m³ for DIA). Thus, there was approximately a factor of 25 separating the smallest from the largest $RD_{50}P$ values.

There was little change in V_T during exposures to MWF "A" or its components. For this reason, it was not possible to develop concentration-response relationships using V_T.

Types of respiratory responses to MWF "A" and its components

As seen in Fig. 1, concentration-dependent decreases in f_R were produced by MWF "A" and its components. These decreases in f_R were due to a combination of sensory and pulmonary irritation. MWF "A" and each component produced both types of respiratory responses. However, the duration of each effect varied, depending upon the airborne chemical to which the animals were exposed. The data are summarized in Table 3.

Sensory irritation

In general, exposure to MWF "A" and its components resulted in immediate sensory irritation. This effect persisted throughout the 3-h exposures with two exceptions, INA and TOL. Sensory irritation faded within approximately 1 h of exposure to INA and very little sensory irritation was seen during the first hour of exposure to TOL.

Table 3 Types of respiratory responses to MWF "A" and its components

Component (concentration range used in this study)	Sensory irritation	Pulmonary irritation	Plateau in response	Recovery immediately post-exposure
AMP [185–1160 mg/m³]	Yes, 0–3 h	Little, 2–3 h	Yes, 2–3 h	Poor
DIA [500–2069 mg/m³]	Yes, 0–3 h	Little, 2–3 h	Yes, 2–3 h	Poor
FAC [107–320 mg/m³]	Yes, 0–3 h	Yes, 2–3 h	Yes, 2–3 h	Moderate
INA [172–755 mg/m³]	Yes, 0–1 h, but fades	Yes, 0–3 h	Yes, 0–1 h	Poor
MIA [230–1005 mg/m³]	Yes, 0–3 h	Little, 2–3 h	Yes, 2–3 h	Moderate-good
PPS [168–639 mg/m³]	Yes, 0–3 h	Little, 2–3 h	Yes, 2–3 h	Poor
SPY [127–630 mg/m³]	Yes, 0–3 h	Little, 2–3 h	Yes, 2–3 h	Very poor
TOL [95–323 mg/m³]	Little, 0–1 h	Yes, 0–3 h	Yes, 1–3 h	Poor
TRI [112–351 mg/m³]	Yes, 0–3 h	Little, 2–3 h	Yes, 1–3 h	Moderate, but deaths within 24–72 h
TIA [329–1070 mg/m³]	Yes, 0–3 h	Yes, 2–3 h	Yes, 2–3 h	Moderate-good
MWF "A"[a] [42–313 mg/m³]	Yes, 0–3 h	Yes, 2–3 h	Yes, 2–3 h	Poor

[a] From Schaper and Detwiler (1991)

Pulmonary irritation

MWF "A" and its components also produced pulmonary irritation in mice. However, this effect was not immediate, but occurred after several hours of exposure. It was more pronounced at higher exposure concentrations than at lower ones, with more consistent and longer pauses occurring between breaths of mice exposed at higher concentrations. Again, the two exceptions were INA and TOL, which evoked marked pulmonary irritation within the first hour of exposure that persisted throughout the remainder of the exposure. By the end of the 3-h exposures when pulmonary irritation was evoked by MWF "A" and its components, there was a plateau in the level of response and this was generally the time at which a maximum response occurred (i.e., maximum decrease in f_R). In some cases (e.g., INA, TOL, TRI), the plateau in response and maximum response was seen earlier in the exposure.

Recovery following exposure to components of MWF "A"

With MWF "A" and the majority of its components (AMP, DIA, INA, PPS, SPY, TOL), f_R failed to return to control levels during the 20-min recovery period immediately following exposure. In fact, with some of these components, there was little increase in f_R after the exposure was terminated. Recovery immediately following exposure was somewhat better with FAC, MIA, TRI, and TIA.

As found by Krystofiak and Schaper (1995), there were delayed deaths of mice after a single 3-h exposure to TRI. The deaths occurred at 24–72 h following exposure to approximately 200 mg/m^3 TRI (i.e., at or above the $RD_{50}P$).

Discussion

As found with MWF "A" (Schaper and Detwiler 1991), its components possessed both sensory and pulmonary irritating properties. On the basis of $RD_{50}P$ values, however, there were differences in their relative potency as irritants. The most potent components of MWF "A" were FAC, TOL, and TRI. With $RD_{50}P$ values of approximately 100–200 mg/m^3, they were similar in potency to one another, and of all components evaluated, most closely matched the potency of MWF "A". The least potent component of MWF "A" was DIA, with an $RD_{50}P$ of 3200 mg/m^3. AMP, INA, MIA, PPS, SPY, and TIA were moderate in potency compared to the other components of MWF "A".

When the fractional concentration (fc) of each component was also considered, it was concluded that FAC and TRI (fc of 0.01–0.1) had much greater contributions to the irritancy of MWF "A" than TOL (fc $<$ 0.01). Thus, of the 11 components in MWF "A", FAC and TRI were identified as the two key irritants. With TRI, there was an additional concern besides sensory and pulmonary irritation, in that delayed mortality was produced. Krystofiak and Schaper (1995) also found delayed deaths following TRI exposure. Given the toxicity of TRI, exposures should be minimized through industrial hygiene control measures to reduce airborne levels (e.g., ventilation, exhaust hoods) or by limiting use of this biocide.

In terms of occupational exposures to MWF "A" and its components, it will be necessary to protect workers from their sensory and pulmonary irritating effects. As proposed by Alarie (1981) and Alarie and Luo (1986), the RD_{50} may be multiplied by 0.03 to obtain an exposure limit that will prevent sensory irritation in the workplace. Indeed, an excellent correlation has been shown between the ACGIH TLV and 0.03 $\times$ $RD_{50}S$ (or 1/30 $\times$ $RD_{50}S$) for 26 chemicals (Alarie 1981), 40 chemicals (Alarie and Luo 1986), and now 89 chemicals (Schaper 1993). If each RD_{50} value given in Table 2 is multiplied by 1/30, then a limit may be obtained *to prevent sensory irritation* from MWF "A" and each of its components. However, as shown in this study, MWF "A" and its components will also produce pulmonary irritation, and this effect must be considered when developing exposure limits. To prevent pulmonary irritation in humans, it has been suggested that the $RD_{50}P$ be mutiplied by 1/60, thus introducing a larger safety factor than that needed for sensory irritants (Weyel et al. 1982; Weyel and Schaffer 1985). Unlike sensory irritants (Schaper 1993), there is not an extensive database for pulmonary irritants and a strong correlation between $RD_{50}P$ values and TLVs has not as yet been established. At the present time, the use of 1/60 $\times$ $RD_{50}P$ serves as a "best guess" for pulmonary irritants.

An occupational exposure limit was calculated for MWF "A" and each of its components by multiplying their respective $RD_{50}P$ values by 1/60. As noted in Table 2, the limits ranged from approximately 2–3 mg/m^3 for MWF "A" and its most potent components, FAC, TOL and TRI, to 50 mg/m^3 for DIA, the least potent component of MWF "A". Recommended exposure limits for the remaining components, AMP, INA, MIA, PPS, SPY, and TIA were approximately 5–14 mg/m^3. These concentrations listed in Table 2 should be acceptable to prevent sensory irritation and should be a good starting point to prevent pulmonary irritation of the components. Future studies may provide additional toxicological or epidemiological data to suggest downward revision of these limits.

Krystofiak and Schaper (1995) have suggested that an exposure limit for a MWF may be predicted from evaluation of the fluid itself or from evaluation of its individual components. In their study of MWF "B" (a semi-synthetic fluid), Krystofiak and Schaper (1995) demonstrated that its components produced the same type of toxic effect (i.e., pulmonary irritation) and used the equation provided by the ACGIH (1994) to predict a TLV for the mixture. The predicted TLV for MWF "B" based on component data was within a factor of 2 of that based on evaluation of the fluid itself (i.e., 5 versus 2.6 mg/m^3).

ACGIH TLV of a mixture:

$$\text{TLV of mixture} = \frac{1}{fc_a/\text{TLV}_a + fc_b/\text{TLV}_b + fc_c/\text{TLV}_c + \ldots fc_n/\text{TLV}_n}$$

where a, b, c, ... n are the components in the mixture.

As noted in Table 1, there were no absolute values for the fractional concentration of each component (fc) of MWF "A". Generally, ranges were listed (e.g., 0.01–0.1). Thus, to determine an occupational exposure limit for MWF "A" on the basis of its components, several assumptions were made. First, it was assumed that fc of AMP, DIA, FAC, INA, MIA, TIA, and TRI was 0.05; a value that was in the middle of the range listed for each of these components. Secondly, it was assumed that fc was 0.005 for PPS, SPY, and TOL; a value that is less than 0.01 as given on the MSDS. Thirdly, it was assumed that SIL did not contribute to the irritancy of MWF "A" and it was excluded in the calculations. Under these conditions, fc for water in MWF "A" would have been 0.63. However, as noted in the Materials and methods section, water was not collected during air sampling in our exposures. Thus, fc was corrected for each component to exclude water. This resulted in fc values of 0.135 for AMP, DIA, FAC, INA, MIA, TIA, and TRI and fc values of 0.0135 for PPS, SPY, and TOL. In Table 2, there are estimates of occupational exposure limits (i.e., possible TLVs to prevent pulmonary irritation) for each component in MWF "A". When these data were used in the above equation, an occupational exposure limit of 6.5 mg/m^3 was obtained for MWF "A". This value was somewhat higher than the limit of 2.0 mg/m^3 for MWF "A" proposed by Schaper and Detwiler (1991). However, it is a reasonable estimate for an exposure limit for MWF "A" and may be refined when there are exact values for fc of each component. Thus, it appeared that the component-based approach used by Krystofiak and Schaper (1995) also worked with MWF "A".

There are obvious advantages and disadvantages in assessing the irritancy of a mixture versus its components. By evaluating individual components, it is possible to identify that (those) which are the most irritating, and this information could allow a manufacturer to modify the fc of components in a formulation or to substitute another chemical that is less irritating. This is clearly an advantage of a component-based approach for evaluating MWFs. However, it is very time-consuming to test each component in a mixture, as done in the present study. Furthermore, when only single chemicals are evaluated, their potential interactions in a mixture may be missed. This did not appear to be the case with MWF "A" (present study) or MWF "B" (Krystofiak and Schaper 1995), since proposed exposure limits were similar whether based on the sum of components or on the fluid itself. This may not always be true for other MWFs and thus, an exposure limit could be overestimated or underestimated if there are chemical interactions of components.

Currently, there is debate as to an appropriate exposure limit for MWFs. For a mineral oil mist, the Permissible Exposure Limit (PEL) set by the US Occupational Safety and Health Administration (OSHA 1989) and the ACGIH TLV (1994) is 5 mg/m^3. This limit is not applicable to MWF "A", since it is a synthetic fluid and contains no oil. It has no relevance to its components. The only guideline that could be applied to MWF "A" or its components comes from the ACGIH (1994) under the category of "particulates not otherwise classified" (PNOC). The TLV is 10 mg/m^3. Based on the results of this study, this will be inadequate to protect workers from the irritating effects of MWF "A" and most of its components. DIA, with our proposed limit of 50 mg/m^3, is perhaps the only component where such a TLV might be reasonable. Thus, TLVs are still needed for MWF "A" and its components. As noted above, the values presented in Table 2 should serve as a starting point.

Finally, previous studies with MWFs (Schaper and Detwiler 1991; Krystofiak and Schaper 1995; Schaper and Detwiler-Okabayashi 1995), as well as the present study, all point to marked variation in the irritating potential of MWFs and their components. They are clearly not a homogeneous group of chemicals. Therefore, it may be difficult to suggest a single TLV for all MWFs and if a single value is to be recommended, then it must take into account the "worst cases" (i.e., those fluids having the greatest toxicity) and may be more conservative than necessary for other fluids.

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ORIGINAL INVESTIGATION

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An approach for evaluating the respiratory irritation of mixtures: application to metalworking fluids

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Abstract Recently, the sensory and pulmonary irritating properties of ten metalworking fluids (MWF) were assessed using a mouse bioassay. Relative potency of the MWFs was estimated, but it was not possible to identify the component(s) responsible for the the respiratory irritation induced by each MWF. One of the ten fluids, MWF "E", produced sensory and pulmonary irritation in mice, and it was of moderate potency in comparison to the other nine MWFs. MWF "E" had three major components: tall oil fatty acids (TOFA), sodium sulfonate (SA), and paraffinic oil (PO). In the present study, the sensory and pulmonary irritating properties of these individual components of MWF "E" were evaluated. Mixtures of the three components were also prepared and similarly evaluated. This analysis revealed that the sensory irritation from MWF "E" was largely due to TOFA, whereas SA produced the pulmonary irritation observed with MWF "E". Both TOFA and SA were more potent irritants than was MWF "E", and the potency of TOFA and/or SA was diminished through combination with PO. There was no evidence of synergism of the components when combined to form MWF "E". This approach for identifying the biologically "active" component(s) in a mixture should be useful for other MWFs. Furthermore, the approach should be easily adapted for other applications involving concerns with mixtures.

Keywords Metalworking fluids · Pulmonary irritation · Mice · Mixtures

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Introduction

For many years, it has been a common practice for toxicologists to evaluate the effects of single chemicals in animal models. In particular, rats and mice have been used to obtain much of the toxicity data found in the scientific literature. Yet, there is a concern with this type of toxicity testing when extrapolating the animal results to humans. In occupational and environmental settings, humans are rarely exposed to only one chemical, but rather to mixtures of chemicals (National Research Council 1988). This is also true for humans exposed while in confined spaces (e.g., airplanes, homes, offices, commercial buildings) which has led to the contemporary, highly-debated issue of indoor air quality. Thus, toxicologists are frequently asked to determine the risks from exposure to multiple chemicals in a variety of settings, and there are no available animal data or limited data on which such projections may be made. This suggests that there is a need for designing studies where animals are also exposed to mixtures, and indeed there has been increasing interest and attention focused on such studies.

Recently, Schaper (1993) prepared a database of chemicals whose sensory irritating properties had been evaluated using a mouse bioassay (Alarie 1966, 1973; American Society for Testing and Materials 1984). Of the 295 airborne materials evaluated with the bioassay, only 51 were mixtures; the remainder were single chemicals. The majority of mixtures were described as "thermal decomposition products" (TDP), indicating that they were generated through heating/combusting of synthetic materials such as polycarbonate, polyurethane, or polyvinylchloride (Barrow et al. 1978; Alarie and Anderson 1979; Sangha et al. 1979; Schaper et al. 1994). In this same review, there were other reports on building materials which included floor

similar to those of PO alone, with evidence of both sensory and pulmonary irritation. However, the two-component mixtures containing SA (i.e., SA + TOFA, SA + PO) evoked pulmonary irritation earlier in the exposures than the mixture that did not contain SA (i.e., TOFA + PO). With each of these mixtures, a plateau in response occurred between the 2nd and 3rd hour of exposure. Recovery was generally poor post-exposure, with the exception of TOFA + PO where some recovery was observed. The irritancy of MWF "E" most closely resembled that of PO alone and TOFA + PO.

Concentration-response relationships

As a result of the sensory and pulmonary irritation, decreases in f occurred in mice exposed to MWF "E", its components, or mixtures of its components. These decreases in f were proportional to the logarithm of the exposure concentration. Concentration-response relationships for MWF "E", its components, and mixtures of its components are illustrated in Fig. 1. As noted in Fig. 1, there was a clustering of the concentration-response curves for SA, TOFA, and the mixtures of SA and TOFA. These curves were located to the left of that for MWF "E". Also, there was a clustering of the concentration-response curves for PO and the mixtures of SA or TOFA with PO which were all located to the right of that for MWF "E".

Using these concentration-response relationships, the RD_{50} values ($RD_{50}S$ or $RD_{50}P$) were determined

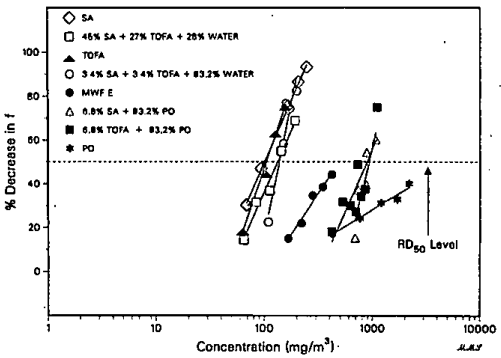

Fig. 1 Concentration-response relationships for MWF "E", its components, and mixtures of components. Each point represents the mean response (i.e.,% decrease in f) of four mice during the 3-h exposures. Linear least squares regression analysis revealed that the slope of each line was significantly different from zero

Table 4 Experimental RD_{50} values, predicted RD_{50} values, and recommended exposure limits for MWF "E", its components, and mixtures of its components

Individual component or mixture of components	Experimental $RD_{50}S$ or $RD_{50}P$ (mg/m^3)	95% confidence interval for $RD_{50}S$ or $RD_{50}P$ (mg/m^3)	Predicted $RD_{50}P$ (mg/m^3)[a]	Recommended exposure limit based upon experimental $RD_{50}S$ or $RD_{50}P$ (mg/m^3)
MWF "E"	497 (P)	453–545 (P)	660–990[b]	8.3
SA	102 (P)	98–105 (P)	–	1.7
TOFA	105 (S)	99–113 (S)	–	3.2[c]
PO	5437 (P)	3603–11483 (P)	–	90.6
45% SA + 27% TOFA + 28% water	134 (P)	120–155 (P)	103	2.23
3.4% SA + 3.4% TOFA + 93.2% water	139 (P)	110–173 (P)	104	2.32
6.8% SA + 93.2% PO	942 (P)	806–1101 (P)	1190	15.7
6.8% TOFA + 93.2% PO	903 (P)	744–1132 (P)	1220	15.1

[a] $RD_{50}P$ values were predicted for mixtures using Eq. 1 (see text). For mixtures containing water, their $RD_{50}P$ values were calculated excluding the water component.
[b] The predicted $RD_{50}P$ was 660 mg/m^3 if the fractional concentrations (c) of SA, TOFA, and PO were assumed to be 0.09, 0.05, and 0.86. However, if the fractional concentrations (c) of SA, TOFA, and PO were assumed to be 0.07, 0.02, and 0.91, then the predicted $RD_{50}P$ was 990 mg/m^3 (see text).
[c] As noted in the text, the main effect of TOFA was sensory irritation and the $RD_{50}S$ was multiplied by 0.03 to arrive at the recommended exposure limit. For all other components or mixtures, there was also pulmonary irritation and $RD_{50}P$ values were divided by 60. Thus, their recommended exposure levels were set to prevent both sensory and pulmonary irritation in humans

for each component and mixture of components. These values are given in Table 4. Because a 50% response level was not achieved with MWF "E" or PO, their $RD_{50}P$ values were extrapolated.

Discussion

This study has demonstrated that the three major components of MWF "E" were capable of producing irritation in mice. However, there were clear differences in the type(s) of irritation which was(were) evoked and the relative potency of the components as irritants. TOFA and SA were similar in potency, but TOFA was predominantly a sensory irritant while SA was a pulmonary irritant. PO was much less potent than TOFA or SA, with over a factor of 50 separating its $RD_{50}P$ value from that of the $RD_{50}S$ for TOFA or $RD_{50}P$ for SA. PO also possessed both sensory and pulmonary irritating properties which distinguished it from TOFA and SA.

With combinations of TOFA, SA, and PO, it was possible to observe alterations in the respiratory irritation of the individual components. This was evident in terms of the type(s) of irritation and/or relative potency. For example, when TOFA was combined with SA in equal proportions, the mixture produced both sensory and pulmonary irritation. Also, the mixture was equivalent in potency to the individual components, which indicated that there was no synergism of the two chemicals. These were not surprising results, in that the properties of the mixture reflected those of the two components. In exposures where TOFA (or SA) was combined with PO (6.8% w/v), sensory and pulmonary irritation were evoked and the potency of the mixtures was less than that of TOFA (or SA), yet greater than that for PO alone. Could this change in potency have been predicted from the individual components? To answer this question Eq. 1 was utilized with the assumption that the respiratory irritancy of TOFA (or SA) with PO was additive. Thus, the RD_{50} for a mixture may be predicted on the basis of its components; 1) when the fractional concentration (c) of each component is known and 2) when the individual $RD_{50}P$ (or $RD_{50}S$) for each component is known.

$$\frac{C_{\text{component 1}}}{RD_{50(\text{component 1})}} + \frac{C_{\text{component 2}}}{RD_{50(\text{component 2})}} + \cdots$$

$$\frac{C_{\text{component n}}}{RD_{50(\text{component n})}} = \frac{1}{RD_{50(\text{mixture})}}$$

For the mixtures containing SA (or TOFA) in PO (i.e., 6.8% SA or TOFA and 93.2% PO), the fractional concentration (c) was 0.068 for SA (or TOFA) and 0.932 for PO. The fractional concentration for each component was divided by its individual RD_{50} value and using Eq. 1, $RD_{50}P$ values for mixtures were predicted (e.g., $1/RD_{50(\text{mixture of SA + PO})} = 0.068/102 + 0.932/5437$). As noted in Table 4, this yielded $RD_{50}P$ values of 1190 mg/m^3 for the mixture of SA in PO and 1220 mg/m^3 for the mixture of TOFA in PO. The experimental $RD_{50}P$ values, which are also given in Table 4, were 942 mg/m^3 for TOFA + PO, and 903 mg/m^3 for SA + PO. Thus, there was good agreement between the predicted and actual $RD_{50}P$ values, and the assumption of simple additivity appeared to be justified.

What conclusions may be drawn regarding MWF "E", the sum of TOFA, SA, and PO? First, MWF "E" possessed both sensory and pulmonary irritating properties, which was not surprising given that it is a mixture of these three major components. Second, the concentration-response relationship and $RD_{50}P$ for MWF "E" fell between those of TOFA (or SA) and PO. As noted in Table 4, the $RD_{50}P$ for MWF "E" was 497 mg/m^3, which on a log scale was approximately mid-way between the RD_{50} for TOFA (or SA) and PO. Using Eq. 1, the $RD_{50}P$ for MWF "E" was also predicted. To perform this calculation, it was assumed that the fractional composition (c) of TOFA, SA, and PO was 9% SA (c = 0.09), 5% TOFA (c = 0.05), and 86% PO (c = 0.86) in MWF "E". These represent the *upper* limits provided on the Material Safety Data Sheet (see Table 1). From Eq. 1, the predicted RD_{50} for MWF "E" would be 660 mg/m^3 (i.e., $1/RD_{50(\text{mixture of SA + PO})} = 0.09/102 + 0.050/105 + 0.932/5437$). Thus, on the basis of these assumptions the predicted $RD_{50}P$ was close to the experimental value of 497 mg/m^3. Even if it was argued that the fractional concentrations for TOFA and SA in MWF "E" were at the *lower* limits given in Table 1 (e.g., $c_{SA} = 0.07$, $c_{TOFA} = 0.02$, $c_{PO} = 0.91$), the predicted $RD_{50}P$ for MWF "E" would be 960 mg/m^3, or within a factor of 2 of the experimental value. This is acceptable agreement when using the mouse bioassay where such variation may be seen while reproducing the RD_{50} (S or P) for a specific chemical in the same laboratory or in different laboratories.

For each component, mixture of components, and MWF "E", occupational exposure limits were determined on the basis of RD_{50} values. As indicated in Table 4, the $RD_{50}S$ value for TOFA was multiplied by 0.03 to arrive at the recommended occupational exposure limit of 3.2 mg/m^3. The use of 0.03 as a "safety factor" to prevent sensory irritation in humans has been demonstrated for several hundred chemicals (Schaper 1993) and is applicable for TOFA. With the remainder of components and mixtures, their $RD_{50}P$ values were divided by a factor of 60 to prevent pulmonary irritation (Weyel et al. 1982; Weyel and Schaffer 1985). The use of 60 as a "safety factor" for pulmonary irritants represents a best guess at the present time and a quantitative relationship between the responses of mice and humans has not been established

as in the case of sensory irritants (Alarie et al. 1980). Thus, the exposure limits given in Table 4 should be considered as upper bounds; additional data (toxicological or epidemiological) may necessitate a lowering of the proposed limits. In practice, it is likely that the safety and health concerns will focus on MWF "E" in the workplace, rather than its components. The current Threshold Limit Value (American Conference of Governmental Industrial Hygienists 1993) or Permissible Exposure Limit (Occupational Safety and Health Administration 1989) for oil mists is 5 mg/m^3. As indicated by the data from our previous study (Schaper and Detwiler 1991) and the present study, this limit appears to be too high for occupational exposures and should be re-evaluated.

The approach used in this study worked very well in defining the irritant properties of components in metalworking fluids, in identifying those which were of greatest potency, and in establishing occupational exposure limits for single chemicals and mixtures. With this information, it should be possible to control workplace exposures to minimize potential irritation. Industrial hygiene "control" may take the form of increased workplace ventilation, but it could also entail re-formulation of a metalworking fluid to remove or decrease the amount of a potent component. For example, SA was a potent component in MWF "E" that produced rapid pulmonary irritation. In future use of MWF "E", it may be useful to replace SA with another component that is less potent and that lacks pulmonary irritating properties.

The approach described here should be easy to apply when evaluating the respiratory irritation of other metalworking fluids, and other types of mixtures where chemical components and their fractional composition are known. Such a detailed evaluation will be time-consuming, but if it is important to know the component(s) in a mixture that is(are) responsible for the irritation of the mixture, then this is a good way to proceed. Also, by taking this approach, it will be possible to identify potential synergism and antagonism of multiple chemicals in a mixture. In the present study, the components behaved in an additive manner which may not be the case with other mixtures.

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Evaluation of the Acute Respiratory Effects of Aerosolized Machining Fluids in Mice

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Evaluation of the Acute Respiratory Effects of Aerosolized Machining Fluids in Mice. SCHAPER, M., AND DETWILER, K. (1991). *Fundam. Appl. Toxicol.* **16,** 309–319. Using a previously developed bioassay, the sensory and pulmonary irritating properties of a group of 10 aerosolized machining fluids were evaluated in mice. Single, 3-hr inhalation exposures were conducted with the fluids at exposure concentrations ranging from 20 to 2000 mg/m^3. The results have shown that all 10 were capable of inducing sensory and pulmonary irritation, with little or no change in pulmonary histopathology. A concentration–response relationship was developed for each fluid which revealed that, for the 10 fluids studied here, the synthetic/semisynthetic and soluble fluids were more potent irritants than the straight oils. Also, 3 of the 10 fluids which had been collected from workplace operations (i.e., "in use" fluids) were found to be similar in potency to the same fluids prior to their introduction into the workplace (i.e., "neat" fluids). From concentration–response relationships, the RD50 value (i.e., concentration inducing a 50% response) was obtained for each of the 10 fluids. The RD50 values ranged from 100 to 1000 mg/m^3 for all fluids except the straight oils whose RD50 values were over 100,000 mg/m^3. Using these values, exposure limits were then suggested for workers in industry to prevent irritation. This bioassay may be a good first step in evaluating new machining fluids whose formulations may change depending upon the current industrial needs.

Millions of gallons of metalworking fluids are used each year by industry for the lubrication of machinery involved in operations such as cutting, milling, drilling, and grinding (Independent Lubricant Manufacturers Association, 1989). In the past, these fluids were primarily petroleum-based oils, often referred to as "cutting fluids" or "cutting oils." Today, a variety of fluids is used, not only for lubrication, but also for cooling of machinery. Three basic categories of machining fluids were first described by Key *et al.* (1983) and recently discussed in a review article by Mackerer (1989). They are (1) straight oils (i.e., insoluble oils), (2) oil emulsions (i.e., soluble fluids), and (3) synthetic/semisynthetic fluids.

A straight oil is typically composed of 60–100% paraffinic or naphthenic oil and is, thus, insoluble in water. These oils may be combined with chlorine or sulfur-based additives when used in high-pressure operations. Soluble machining fluids, in their concentrated form, contain 30–85% (v/v) oil along with emulsifiers, corrosion inhibitors, defoamers, dyes, water conditioners, and other additives. The concentrate is then diluted with water (e.g., 2–20% v/v) prior to usage. It is the presence of this large proportion of water (i.e., 80–98%) that makes these fluids well-suited as coolants. It is common practice to add a biocide to such fluids to prevent bacterial growth. Synthetic fluids contain no oil in their formulations, whereas semisynthetic fluids contain approximately 5–30% (v/v) oil. Corrosion inhibitors

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0272-0590/91 $3.00

and surfactants are found in both of these types of fluids. Other additives such as dyes, water conditioners, and defoamers may be present in the synthetic and semisynthetic fluids. Further dilution of synthetic/semisynthetic fluids with water may be conducted in the workplace and, like the soluble machining fluids, biocides may be added due to their high water content.

Since the 1950s, there has been increasing concern regarding the effects of machining fluids on the skin and lungs of exposed workers. These workers may come into direct contact with the fluids, creating the potential for percutaneous absorption. Of greater importance are the mists which are produced by the high pressure and temperature encountered during machining operations (Costa and Amdur, 1979; Mackerer, 1989; Chan *et al.*, 1990). These mists may be deposited on the skin and absorbed through it. Furthermore, they may be inhaled. With the small aerosol that is formed (i.e., mass median aerodynamic diameter below 10 μm) (Ayer, 1964; Kennedy *et al.*, 1989; Chan *et al.*, 1990), significant deposition may occur in the respiratory tract. Also, workers are likely to be exposed to these mists each day over a period of years. Thus, the potential exists for adverse acute and chronic pulmonary effects to be induced by them.

A number of epidemiology studies (Christian, 1962; Decoufle, 1976, 1978; Goldstein *et al.*, 1970; Hendricks *et al.*, 1962; Jarvholm *et al.*, 1981; Jarvholm and Lavenius, 1987; Ronneberg *et al.*, 1988; Vena *et al.*, 1985; Waldron, 1975) evaluated lung (and other vital organ) cancer mortality and morbidity in workers exposed to machining fluids (generally, straight oils). It is not surprising that the investigators reached different conclusions, given that "exposure" varied greatly in terms of exposure concentrations, duration of exposure, and types of straight oils used. Thus, it appears that the question of increased lung cancer risk among workers exposed to machining fluid mists is still debatable. Other investigators (Ely *et al.*, 1970; Jarvholm, 1982; Kennedy *et al.*, 1989; Oxhoj *et al.*, 1982) have examined pulmonary function and respiratory symptoms in workers exposed to machining fluid mists. Both Jarvholm (1982) and Oxhoj *et al.* (1982) conducted epidemiology studies which showed an increased prevalence of chronic cough, chronic phlegm, and dyspnea among machine shop workers. However, they found no decrement in lung function as determined via spirometry. Ely *et al.* (1970) also found no decrease in lung function among machine shop workers, but they did not find an increase in respiratory symptoms as did Jarvholm (1982) and Oxhoj *et al.* (1982). In a recent study by Kennedy *et al.* (1989), pulmonary function measurements were performed pre- and postshift on Mondays and Fridays in workers exposed to mists of straight oils, soluble fluids, or synthetic fluids. Cross-shift reductions in FEV_1 occurred on both Mondays and Fridays without any additional decrement in FEV_1 over the week. These changes were attributed to acute airway obstruction as induced by machining fluid mist whose concentration was above 0.20 mg/m^3 and whose droplet size was $\leq$9.8 μm. The results were consistent among the worker population studied, regardless of the type of fluid to which each was exposed.

In terms of animal studies, few inhalation exposures have been conducted. Nearly 40 years ago, Lushbaugh *et al.* (1950) and Shoshkes *et al.* (1950) were interested in histopathological changes in the lungs of animals (i.e., mice, rabbits, rats, monkeys) repeatedly exposed to oil mists. Lushbaugh *et al.* (1950) aerosolized automobile lubricating oil and diesel engine lubricating oil at concentrations between 63 and 132 mg/m^3. Shoshkes *et al.* (1950) worked with a larger variety of oils, including peanut, corn, cod liver, mineral, and motor oils, and used higher exposure concentrations (e.g., 4300–12600 mg/m^3). Both groups of investigators reported rapid increases in the number of macrophages in the lungs of exposed animals immediately following exposure. Within 48 hr of a short exposure (e.g., 2 hr), phagocytosis of oil droplets was completed. In 1964, Wagner *et al.* exposed mice,

rats, hamsters, rabbits, and dogs to mineral oil mist at 5 or 100 mg/m^3 for a period of 12–26 months. They found no impairment of pulmonary function as assessed by measurements of oxygen consumption and minute ventilation. Like Lushbaugh *et al.* (1950) and Shoshkes *et al.* (1950), Wagner *et al.* (1964) also found increases in numbers of pulmonary macrophages in exposed animals.

In more recent years, studies were conducted by Stula and Kwon (1978), Costa and Amdur (1979), and Selgrade *et al.* (1990). Like the earlier studies, Stula and Kwon (1978) also evaluated histopathological changes in the lungs of dogs, rats, mice, and gerbils exposed to a mineral oil-based mist at concentrations of 5 or 100 mg/m^3. Their results were similar to those of Wagner *et al.* (1964). Costa and Amdur (1979) were the first investigators to conduct pulmonary function measurements in guinea pigs during exposure to aerosols of straight oils. Their exposure concentrations ranged from 100 to 200 mg/m^3. They found little, if any, change in respiratory frequency or tidal volume. However, at mist concentrations above 200 mg/m^3, decreases in lung compliance occurred. Selgrade *et al.* (1990) exposed rats to a light-weight lubricating oil (i.e., straight oil) that was vaporized and then condensed so as to produce an aerosol. Rats were exposed to the aerosol for 3.5 hr/day for 4 days/week over a 13-week period. Some animals were examined 1 day after these repeated exposures, while others were examined following a 4-week recovery period. Little change in pulmonary histopathology or pulmonary function was found in these animals, even at the highest exposure concentration used, 1500 mg/m^3.

No attempt has been made in any previous epidemiological or toxicological study to propose exposure limits for workers who use metalworking fluids. Currently, the permissible exposure limit (PEL) (U.S. Department of Labor, 1989) and the threshold limit value–time-weighted average (TLV-TWA) (American Conference of Governmental Industrial Hygienists, 1986, 1989) for an oil mist (mineral) is set at 5.0 mg/m^3. This would be applicable for straight oils, but not for the newer types of machining fluids that contain little or no oil (i.e., synthetics/semisynthetics). For mists produced from these fluids, the current PEL would be 5.0 mg/m^3, respirable fraction, or 15.0 mg/m^3, total. These values are given under the category, "particulates not otherwise regulated."

As described above, a much larger variety of machining fluids is commercially available today than ever before. Thus, the present study was undertaken to evaluate the acute respiratory effects from a group of machining fluids using a previously developed mouse bioassay (Alarie, 1966, 1973, 1981; Alarie and Luo, 1986). With this approach, the fluids may be rapidly screened for irritation potential. Furthermore, if concentration–response relationships are developed, then comparison of their biological potency would be possible. This has not been done to date. Using the responses obtained in mice, exposure limits may then be suggested to prevent irritation in man and comparisons with current PELs and TLV-TWAs may be made.

METHODS

Animals

Specific pathogen-free, male, Swiss–Webster mice were used for all exposures. These animals were obtained from Hilltop Lab Animals (Scottdale, PA). They were housed with food (Purina Chow) and water *ad libitum* in an animal room on a 12-hr dark/light cycle. A new group of four mice was employed in each experiment.

Machining Fluid Samples

A total of 10 machining fluids was supplied to the University of Pittsburgh by the United Auto Workers (UAW) and General Motors (GM) National Joint Committee on Health and Safety. They were obtained from three GM plants: (1) the Hydramatic Division in Ypsilanti, Michigan, (2) the Saginaw Division in Detroit, Michigan, and (3) the Saginaw Division in Saginaw, Michigan. All samples were shipped on dry ice and delivered to the University of Pittsburgh within 24 hr of shipment. The fluids were kept in

a cold room (approximately 5°C) until they were needed for exposures.

Of the 10 fluids, 7 fluids had not as yet been introduced into workplace operations (i.e., "neat" fluids). Formulations of the 7 neat fluids are given in Table 1. None of these fluids was diluted with water but it is apparent that the synthetic/semisynthetic fluids (i.e., Samples A and B) contained much larger amounts of water in their formulations in comparison to the other neat fluids.

The three "in use" fluids were obtained from workplace operations involving metal cutting, grinding, or cooling and they are designated as Samples B', E', and F'. Samples B' (in use semisynthetic fluid) and E' (in use soluble fluid) were dilutions (typically 5% v/v) of the respective neat fluids, B and E, and thus contained larger proportions of water than these fluids. On the other hand, Sample F' (in use straight oil) may have contained contaminants not present in Sample F (neat straight oil) but there was no dilution with water since it was a straight oil. Further chemical analysis was not conducted here to determine other possible differences in composition between in use and neat fluids.

All neat and in use machining fluids were used *as received* (i.e., no dilution or other alterations) and aerosols were generated from these fluids at room temperature.

Generation of Test Exposures

Using a Harvard Apparatus syringe pump, each machining fluid was fed into a Pitt No. 1 aerosol generator (Wong and Alarie, 1982). For a small number of experiments, a Pitt No. 4 aerosol generator (Rosato *et al.*, 1988) was used when attempting to generate high exposure con-

TABLE I

FORMULATION OF THE SEVEN NEAT MACHINING FLUIDS BASED ON MATERIAL SAFETY DATA SHEETS

Neat Sample A: Synthetic fluid
- 1–10% Fatty acid–alkanolamine condensates
- 1–10% 2-Amino-2-methyl-1-propanol
- 1–10% Isononanoic acid
- 1–10% Monoisopropanolamine
- 1–10% Diisopropanolamine
- 1–10% Triisopropanolamine
- 1–10% Preservatives
- <1% Phosphonate sequestrant
- <1% Tolutriazole
- <1% Defoamer
- >60% Water

Neat Sample B:[a] Semisynthetic fluid
- 10% Alkanolamide
- 5% Boramide
- 20% Petroleum oil
- 3% Sodium sulfonate
- 54% Water
- 3% Triazine
- 5% Potassium soaps

Neat Sample C: Soluble fluid
- <2% SDA 23-099-00
- 80% Solvent refined heavy paraffinic distillate (petroleum)
- 7–9% Tall oil
- <3% Oxybispropanol
- 3–5% Sodium sulfonate
- 1–2% Potassium hydroxide
- 0.2–1% 100 Neutral naphthenic oil
- Balance % water

Neat Sample D: Soluble fluid
- 70–90% Saginaw division reclaimed oil (mineral oil)
- 15–25% Sulfonic acids, petroleum, sodium salts
- 15–25% Tall oil fatty acids, potassium salt
- 15–25% 1,2-Propanediol
- <2% Microcrystalline wax (petroleum)
- 1–2% Phosphoric acid, 2-ethylhexyl ester
- 2–7% Glycine, *N*-(C13-18-alkylsulfonyl) derivatives, compounds with triethanolamine

Neat Sample E:[a] Soluble fluid
- <30% Tall oil fatty acids, potassium salt
- <30% Petroleum sulfonic acid (petroleum)
- Balance % catalytic dewaxed light paraffinic oil (petroleum)

Neat Sample F:[a] Straight oil
- 100% Sulfonized mineral oil

Neat Sample G: Soluble fluid
- 70–90% Hydrotreated heavy naphthenic distillate (petroleum)
- 10–20% Sulfonic acids, petroleum, sodium salts
- 10–20% Tall oil fatty acids, potassium salt
- 10–20% 1,2-Propanediol
- <2% Microcrystalline wax (petroleum)

[a] Three in use samples (i.e., collected from machining operations), were also tested in the bioassay. The in use fluids have been designated as B', E', and F'. Their exact formulations are not known but are presumed to resemble those of the original neat fluids, B, E, and F, respectively (see text).

centrations. The rate of fluid delivery to the generator varied from approximately 0.03 to 3.0 ml/min. Dried, compressed air (70–140 kPa) also passed into the generator, resulting in mist formation as fluid reached the jet of the generator. The output of the Pitt No. 1 or No. 4 generator was approximately 10 liters of air/min and was directed into the mouse exposure chamber (described below).

To determine mist concentration during each exposure, air samples were drawn from the mouse exposure chamber onto Gelman, Type A/E glass fiber filters (47 mm diameter, Gelman Sciences, Inc., Ann Arbor, MI). Gravimetric analysis was conducted for all samples using a Mettler balance (Model AE240, Mettler Instrument Corp., Hightstown, NJ). An Andersen mini-impactor or a Marple personal cascade impactor (Model 290, Andersen Samplers Incorporated, Atlanta, GA) was used for sizing the aerosols.

It is important to recognize that the gravimetric analysis reflected only the solid content and low vapor pressure components of the machining fluids. Although many fluids also contained significant proportions of water and other components having a high vapor pressure, the air used for aerosolization and dilution permitted complete vaporization of these components and thus they were not retained by the filters. Therefore, the variable water content did not influence the exposure concentrations given under Results.

Measurement of Animal Respiration and Length of Exposure

Each mouse was positioned in a body plethysmograph so that only its head protruded into the interior of the exposure chamber (Barrow *et al.*, 1977). This chamber was made of glass and had a volume of 2.5 liters. Four body plethysmographs were attached to it as previously described (Barrow *et al.*, 1977). This chamber was continuously ventilated at a rate of 20 liters/min. Attached to each body plethysmograph was a sensitive pressure transducer (Gaeltec 8T-2, Hackensack, NJ) which permitted the measurement of plethysmographic pressure changes as created with each breath. The output of these transducers was directed into a Gould 4-channel recorder. With this arrangement, the pressure changes due to respiration of the four mice were continuously monitored. All signals (analog) were digitized at a rate of 200 samples/sec using a Metrabyte analog to digital converter (Model DAS-16) and stored on a Trillian Power Systems personal computer (Model II, 386 chip).

The amplitude of the plethysmographic pressure changes, corresponding to thoracic displacement, was taken as tidal volume (VT). Calibration of VT was done using a Harvard Apparatus small animal ventilator which delivered a known volume at a known frequency into (and out of) the plethysmograph. This ventilator was previously calibrated using a pneumotachograph. However, it was not critical to obtain absolute values for VT since only relative changes in VT were of interest here. Respiratory frequency (f) was also obtained for each of the four mice by counting the number of pressure waves per unit time. This was done every 15 sec and displayed on a video terminal. Following exposure, VT and f were plotted as a function of time for each mouse. Also, mean tidal volume ($\overline{VT}$) of the four mice (± 1 standard deviation) and mean respiratory frequency ($\bar{f}$) of the four mice (± 1 standard deviation) were plotted as a function of time.

Each experiment was 220 min with a 20-min control period, a 180-min exposure, and a 20-min recovery.

Recognition of Sensory and/or Pulmonary Irritation Response

The plethysmographic pressure changes of the four exposed mice were continuously monitored throughout each experiment, which enabled immediate identification of changes in their breathing patterns. As described previously (Alarie, 1973, 1981), characteristic changes in respiratory patterns are observed during exposure to sensory and pulmonary irritants in mice or in other species. With sensory irritants (i.e., chemicals capable of stimulating trigeminal nerve endings in the nasal mucosa), a lengthening of the expiratory phase of each breath occurs in mice. With pulmonary irritants (i.e., chemicals capable of stimulating vagal nerve endings), a pause occurs between breaths in mice (Alarie, 1981; Schaper *et al.*, 1989). Both sensory and pulmonary irritants will evoke a decrease in f where the level of decrease in f is proportional to exposure concentration (Alarie, 1981).

Statistical Analysis

The maximum change in f that occurred during the 180-min. exposure was evaluated with respect to control. A t test (Armitage, 1977) was used to test for significant responses ($p < 0.05$). When significant responses were found, they were examined as a function of the logarithm of exposure concentration. Least-squares regression analysis was then conducted to establish concentration–response relationships (i.e., testing that the slope of the line from regression analysis was significantly different from zero, $p < 0.05$). Also, these relationships were used to calculate the exposure concentration resulting in a 50% decrease in respiratory frequency (RD50) of the exposed animals.

Histopathology

The lungs were removed from 132 mice, representing 33 groups of animals. Of this number, 120 mice were exposed to machining fluid mists at approximately the RD50 concentrations, while the remaining 12 were the controls. Of the RD50-exposed animals, lungs were removed from

40 mice immediately following exposure to the mists. At 24 hr postexposure, lungs were removed from another 40 mice. At 14 days postexposure, the lungs were removed from the final group of 40 mice. The controls were treated similarly, with the removal of lungs from 12 animals, 4 immediately following exposure to room air, 4 at 24 hr, and 4 at 14 days postexposure to room air.

These lungs were then inflated at 20 cm H_2O with 10% (v/v) buffered formalin for 2 hr followed by immersion in formaldehyde solution for further fixation. For all these exposed and control animals, lung weight and lung volume displacement were also measured prior to and following the inflation with formalin. Slides of sectioned lung were examined following staining with hemotoxylin and eosin.

RESULTS

Sensory irritation was evoked immediately upon exposure to the mists generated from Samples A, B, E, E', and G and it was observed throughout the 3-hr exposures. In addition, pulmonary irritation occurred after approximately 2 hr of exposure. Thus, both sensory and pulmonary irritation patterns were noted at the end of the 3-hr exposures. For the remaining five fluids, B', C, D, F, and F', sensory irritation was also evoked immediately upon exposure to the mists but it tended to fade within 1 hr and often sooner. Pulmonary irritation was then observed after approximately 2 hr of exposure and became more pronounced by the end of the 3-hr exposures.

f decreased rapidly upon exposure to all mists, reaching a plateau after approximately 2 hr. Following exposure to the mists, recovery (i.e., return of f to control levels) was prompt in animals exposed at lower concentrations. As exposure concentration was increased, however, slower recovery was seen immediately following exposure.

VT did not change during the majority of exposures to aerosolized machining fluids. Only at very high exposure concentrations where f decreased by some 70–80% were significant reductions in VT also seen. Here, VT decreased by 30–50% and little recovery occurred postexposure. Thus, no concentration–response relationship was established for VT.

As shown in Fig. 1, concentration–response relationships using f were developed for all 10 machining fluids tested. From these curves that concentration capable of inducing a 50% reduction in f (i.e., RD50) was determined. For 4 fluids, it was necessary to extrapolate the RD50 since it was not possible to produce exposure concentrations at or above the RD50 with the aerosol generators employed here. This situation occurred with Samples B', E', F, and F'. The highest exposure concentration generated for each of these 4 samples is also given in Table 2.

At the RD50 concentration (or the highest achievable concentration for the 4 samples mentioned above), particle sizing was done. The results of these analyses are shown in Table 2. Most machining fluid mists had a mass median aerodynamic diameter (MMAD) of approximately 2.0 μm, with the exceptions of Samples D and E that were larger, at 6.6 and 4.4 μm, respectively. The geometric standard deviation (σ_g) for each mist was approximately 2.0, with little variation between the 10 samples.

The lungs of animals exposed to the RD50 concentration (or as close to this level as possible) were evaluated for changes in lung weight and lung volume displacement. Little change was seen for either measurement. Also, these lungs were evaluated for histopathological changes. The most significant lesions were found at 24 hr postexposure, with little difference in exposed animals from the controls immediately postexposure and at 14 days postexposure. At 24 hr following exposure, mild to moderate interstitial pneumonitis and bronchopneumonia were observed, although not consistently in all animals evaluated. This finding was most prominent in mice exposed to Sample C. Mild interstitial pneumonitis was seen in animals exposed to Samples B, B', E, E', F, F', and G. Little, if any, histopathological changes occurred in the lungs of mice exposed to either Sample A or Sample D.

DISCUSSION

The results of this study have shown that the 10 aerosolized machining fluids possessed

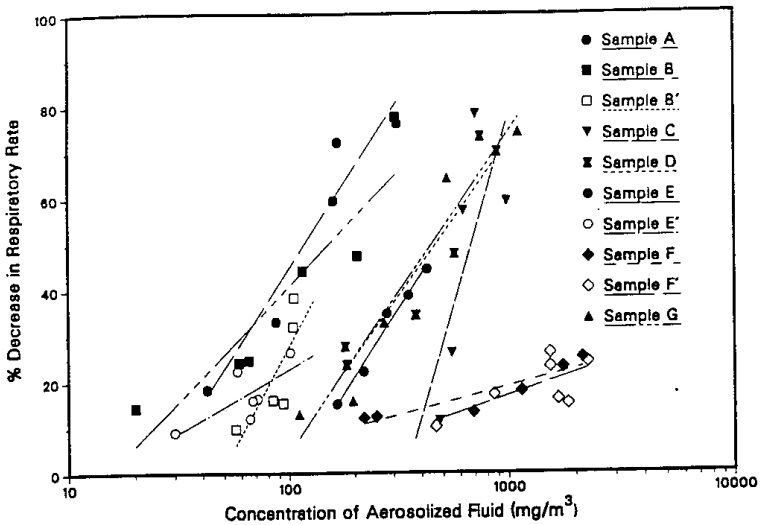

FIG. 1. Concentration–response relationships for the 10 aerosolized machining fluids. Each point represents the mean response of four mice during the 3-hr exposure. The "percentage decrease" was calculated from the preexposure *f* value for each group and the minimum *f* value for each group during the 3-hr exposure. Linear regression analysis was conducted using the data points obtained with each fluid. The slopes of all lines shown here were significantly different from zero.

both sensory and pulmonary irritating properties. The main effect of these fluids was on *f*, with little change in VT. Initially, sensory irritation was induced which resulted in immediate reductions in *f*. Later, in these same exposures, pulmonary irritation occurred which was largely, if not entirely, responsible for the decreases in *f*. For some fluids, sensory irritation occurred throughout the exposure with pulmonary irritation also occurring toward the end of exposure. Thus, both effects contributed to the reductions in *f*. It is unclear at this time why some fluids produced sensory irritation throughout the 3-hr exposure, while with other fluids there was a fading of the sensory irritation response.

The results obtained here in the exposures of mice to straight oils (i.e., Samples F and F') agreed with previous findings of Costa and Amdur (1979). They also found little change in *f* or VT in guinea pigs exposed to straight oils and thus concluded that the mists were not irritating. This then suggests that the irritation observed here with the remaining machining fluids (i.e., synthetic/semisynthetic and soluble fluids) was probably due to differences in chemical composition.

Little change in lung weight, lung volume displacement, or pulmonary pathology was noted immediately following exposure to any machining fluid. Only at 24 hr postexposure was there evidence of mild to moderate interstitial pneumonitis and bronchopneumonia. This supported the observed respiratory responses, showing some evidence of pulmonary irritation. However, these fluids did not produce the extensive hemorrhage and edema seen, for example, with the potent pulmonary irritant, methyl isocyanate (Ferguson *et al.*, 1986). The lack of extensive pathological

TABLE 2

RD50 VALUE AND PARTICLE SIZE FOR THE 10 AEROSOLIZED MACHINING FLUIDS

Fluid sample	Type of fluid	RD50[a] (mg/m^3)	MMAD (μm)	σ_g	Suggested exposure limit (mg/m^3)[b]
1. Sample A	Neat, synthetic	119 (82–171)	2.2	2.1	2.0
2. Sample B	Neat, semisynthetic	154 (92–260)	1.4	1.5	2.6
3. Sample G	Neat, soluble	452 (302–679)	1.5	1.6	7.5
4. Sample D	Neat, soluble	472 (342–652)	6.6	2.1	7.9
5. Sample E	Neat, soluble	497 (453–545)	4.4	1.7	8.3
6. Sample C	Neat, soluble	683 (481–985)	1.6	1.7	11.4
7. Sample B'	In use, semisynthetic	181[c,d]	2.2	2.0	3.0
8. Sample E'	In use, soluble	1,017[c,d]	1.5	2.0	17.0
9. Sample F'	In use, straight oil	110,100[c,d]	2.6	2.0	>1000
10. Sample F	Neat, straight oil	325,000[c,d]	2.7	2.1	>1000

[a] Ninety-five percent confidence intervals are given in parentheses following each RD50 value.

[b] Exposure limits *to prevent irritation* were suggested by dividing the RD50 by a factor of 60. However, no exposure above 5.0 mg/m^3 is permitted by OSHA for oil mists or respirable particulates not otherwise regulated (see text).

[c] Extrapolated RD50 value and no 95% confidence intervals are given.

[d] Highest concentration that was generated: Sample B', 113 mg/m^3; Sample E', 81 mg/m^3; Sample F', 2816 mg/m^3; Sample F, 2492 mg/m^3.

changes in machining fluid-exposed mice is consistent with the results of Lushbaugh *et al.* (1950), Shoshkes *et al.* (1950), Wagner *et al.* (1964), Stula and Kwon (1978), and Selgrade *et al.* (1990).

With the bioassay used in this study, it was evident that there were differences in biological potency between the 10 machining fluids. From the RD50 values presented in Table 2, the 2 most potent fluids were Samples A and B. Sample B' (in use fluid) was similar in potency to Sample B (neat fluid). Next in potency were Sample G, Sample D, Sample E, and Sample F. Sample E' (in use fluid) was slightly less potent than Sample E (neat fluid). The least potent machining fluids were Samples F and F' (neat vs in use straight oil). In more general terms, these data have shown that the 3 synthetic/semisynthetic fluids were somewhat more potent than the 5 soluble fluids. However, the 3 synthetic/semisynthetic and 5 soluble fluids were far more potent than the 2 straight oils. For the 3 in use fluids tested here, there were no significant differences between potency of each in use fluid versus the corresponding neat one. This finding does not imply, however, that other sets of neat and in use fluids will be of equivalent potency to one another. Furthermore, it cannot be concluded for all machining fluids that the relative order of potency will be synthetic/semisynthetic > soluble > straight. A much larger data base would be needed to validate such a ranking.

From this study, it was not possible to precisely determine the component(s) in each machining fluid responsible for the respiratory effects induced in mice. Kennedy *et al.* (1989) also reached a similar conclusion in their study of respiratory effects of machining fluid mists in automotive workers. However, the material safety data sheets as prepared by the manufacturers can be used to provide some suggestions to explain the experimental findings. Clearly, the synthetic/semisynthetic fluids were complex mixtures, containing many potential irritants. For example, Sample A, a synthetic fluid, contained a number of amines (e.g., monoisopropanolamine, diisopropanolamine, etc.). Gagnaire *et al.* (1989), Nielsen and Vinggaard (1988), and Vinggaard *et al.*

(1989) have previously studied a variety of amines and found that they induced both sensory and pulmonary irritation in mice. Thus, it is likely that the considerable proportion of amines (i.e., up to 30% v/v) in Sample A contributed to the observed effect. The neat soluble fluids in their concentrated form were composed of a substantial proportion of petroleum oil (i.e., 60–90% v/v), but each fluid also had numerous other additives. With the presence of these additives, mice were again exposed to complex mixtures. Triethanolamine, an additive in Sample D, would be expected to produce sensory and pulmonary irritation based on previous studies with amines (Gagnaire *et al.*, 1989; Vinggaard *et al.*, 1989). Phosphoric and sulfonic acids, also present in Sample D, would be expected to produce irritation, similar to that reported for sulfuric acid (Wong and Alarie, 1982), although their potency should be lower. Thus, the additives present in soluble fluids, like those present in synthetic/semisynthetic fluids, are important in determining their biological potency as irritants. This is apparent upon comparison of either of these types of fluids with the two straight oils (i.e., F and F') which were entirely composed of petroleum oil with few additives. The straight oils tested here were clearly the least potent.

With regard to exposed workers, the animal bioassay employed in this study can be used to suggest occupational exposure limits to prevent irritation. This extrapolation from mice to men has been previously described at length. Briefly, the RD50 is multiplied by 0.03 to suggest occupational exposure limits for sensory irritants (Alarie and Luo, 1986), while for pulmonary irritants, the RD50 is divided by 60 (Weyel *et al.*, 1982; Weyel and Schaffer, 1985). Because all 10 machining fluids produced pulmonary irritation, the latter factor was applied to each RD50 and the results are given in Table 2. The current OSHA PEL (U.S. Department of Labor, 1989) and TLV-TWA (American Conference of Governmental Industrial Hygienists, 1989) for an oil mist is 5.0 mg/m^3. Likewise, the PEL for "respirable particulates not otherwise regulated" is 5.0 mg/m^3. This is slightly higher than the exposure limits suggested for Sample A and Samples B and B' (synthetic/semisynthetic fluids), but is slightly lower than the exposure limits proposed for Samples C, D, E, E', and G (soluble fluids). The OSHA PEL and ACGIH TLV-TWA for oil mists is over 100 times lower than exposure limits proposed for Samples F and F'. Thus, the current PEL and TLV-TWA should be adequate to protect workers from the acute irritant effects of Samples F and F' (straight oils) but may not provide adequate protection from the irritant effects of A, B, B', C, D, E, E', and G (soluble and synthetic/semisynthetic fluids).

It is important to remember that this study has only evaluated the respiratory effects of single, 3-hr exposures. In occupational settings, workers may be exposed to these airborne materials daily and yearly. Thus, as shown by Hendy *et al.* (1985) and Kennedy *et al.* (1989), there may be adverse pulmonary effects in workers continuously exposed to machining fluid mists at concentrations lower than the PEL and TLV-TWA. Certainly, there is a potential for cumulative or delayed pulmonary effects, neither of which has been examined here. Thus, revision of current PEL and TLV-TWA may be appropriate, pending future studies.

When suggesting exposure limits for humans to prevent irritation from machining fluid mists, another important point to bear in mind is that airborne exposure concentrations are generally assessed via filter sampling and gravimetric analysis. With the large proportion of water that is present in the soluble and synthetic/semisynthetic fluids, water mist and water vapor will be formed in the workplace. As stated under Methods, this did not present a problem for the laboratory study here in mice. However, in the workplace where conditions are not carefully controlled as in the laboratory, greater caution must be taken when conducting gravimetric analysis. One suggestion is to desiccate filters, thus avoiding errors in reporting exposure concentration.

Also, with many of the newer types of machining fluids, a variety of additives is included in their formulation. Many of these additives, such as amines, have a reasonably high vapor pressure and will be volatilized during plant operations. By conducting filter sampling, these airborne materials will not be captured. Thus, it may be appropriate to conduct other forms of exposure assessment for these machining fluids.

In summary, the bioassay employed in this study has yielded results which permitted comparisons of potency of aerosolized machining fluids to be made. Here, a variety of fluids which are currently utilized in automotive plants was evaluated. It was possible with the bioassay to compare potency of neat and in use samples. However, this bioassay could also be used to evaluate newly formulated machining fluids prior to their introduction into the workplace. For the straight oils studied here, the current PEL of 5.0 mg/m^3 may provide adequate respiratory protection for workers, but based upon the soluble and synthetic/semisynthetic machining fluids studied here, the PEL may need to be revised downward following extended exposure studies.

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