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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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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 1

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

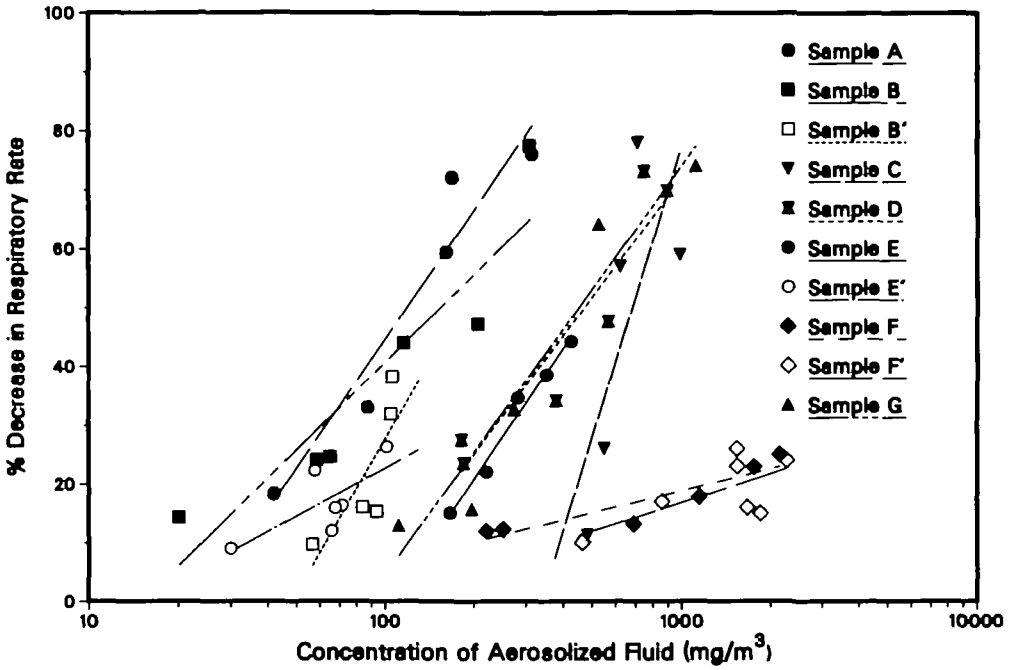

FIG. 1. Concentration–response relationships for the 10 aerosolized machining fluids. Each point represents the mean response of four mice during the 3-hr exposure. The "percentage decrease" was calculated from the preexposure *f* value for each group and the minimum *f* value for each group during the 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/m³)	MMAD (μm)	σ_g	Suggested exposure limit (mg/m³)[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/m³ 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/m³; Sample E′, 81 mg/m³; Sample F′, 2816 mg/m³; Sample F, 2492 mg/m³.

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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