FINAL PERFORMANCE REPORT

" Pulmonary Effects of Machining Fluid Aerosols"

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I. Significant Findings

A. The relative toxicity of 3 major classes of machining fluids (soluble, semi-synthetic, and synthetic) was compared in an animal model of pulmonary injury and inflammation. Significant differences in toxicity were observed in guinea pigs after a single 3 hour exposure to respirable aerosols of unused machining fluids (semi-synthetic > soluble >> synthetic). These findings suggest that the various types of machining fluids may have inherent differences in toxicity in the workplace.

B. Greater toxicity was observed in guinea pigs exposed for 3 hours to used machining fluid aerosols compared to aerosols of unused fluids. Significant changes in biochemical and cellular parameters occurred in bronchoalveolar lavage fluid after a single exposure to 5 mg/m^3 of a used, but not unused, machining fluid aerosol. The observed increase in toxicity of the used machining fluids aerosols was correlated with the airborne concentration of contaminating endotoxin. Thus, microbial contamination of 'in use' machining fluids can also contribute to the pulmonary toxicity of machining fluid aerosols.

C. Physico-chemical properties of machining fluids were also found to contribute to adverse pulmonary effects. Adjustment of either the alkalinity or the hypotonic nature of an unused semi-synthetic machining fluid (to pH 7 and isotonicity, respectively) significantly reduced pulmonary injury and inflammation in guinea pigs. Together with the results presented above, these findings strongly suggest that multiple factors contribute to the adverse respiratory effects associated with acute occupational exposure to machining fluid aerosols.

D. Little to no pulmonary injury or inflammation was produced in guinea pigs exposed to 5 mg/m^3 used machining fluid aerosols for 30 days. The only significant increase in lavage parameters was a 5-fold increase in PMNs. This finding suggests that adaptation to most of the adverse pulmonary effects, which are observed after a single exposure, can develop upon repeated exposure to machining fluid aerosols.

E. Three daily exposures of rats to 10 mg/m^3, but not 1 mg/m^3, used (endotoxin-contaminated) machining fluid aerosols produced a significant increase in stored mucosubstances in the epithelial lining of the intrapulmonary airways and the nasal septum. These changes in stored mucosubstances were accompanied by a significant increase in total cells and neutrophils in the lavage fluid. Surprisingly, a significant increase in stored mucosubstances was observed in the nasal septum, but not the intrapulmonary airways, of animals exposed to 10 mg/m^3 <u>unused</u> machining fluids (no measurable endotoxin). These results suggest that in addition to endotoxin, irritant components of machining fluids can

contribute to the increase in sputum and chronic bronchitis reported for workers exposed to machining fluid aerosols.

II. Usefulness of Findings

Little is known about the agent(s) responsible for the adverse effects or the potential for chronic injury from repeated exposure to the oil mists. The complexity of machining fluid aerosols is great: the solutions contain one of a variety of oils (e.g., straight, soluble, or synthetic) as well as biocides and other additives. The solutions are generally alkaline (approximately pH 9), frequently hypoosmolar, and may be contaminated with a wide variety of bacterial and fungal agents. Therefore, evaluation of the potential for this complex aerosol to cause acute or chronic respiratory effects is dependent on the individual contributions of the components as well as their possible interactions. Identification of the oil/water component(s) responsible for the adverse health effects associated with machining fluid aerosol exposure is paramount in importance for providing the workers with a healthy work environment.

This research project has demonstrated that the injury and inflammation which follow acute exposure to machining fluid aerosols can be dependent on many factors inherent in the machining fluid to which workers are exposed. These factors include the type of machining fluid (i.e., synthetic, semi-synthetic, or soluble), the level of microbial contamination of the fluid, and physico-chemical properties such as pH and osmolarity. Thus, end users of machining fluids must make several decisions in their choice of the safest fluids to use in the lubrication and cooling of their machining operation. Most importantly, and not surprisingly, the end users must ensure that the machining fluids are kept as free of microbial contamination as possible. This latter aim is typically accomplished with the use of biocide, which because of their very nature, must be used with caution as they will also be inhaled as part of the machining fluid aerosols.

This research project also examined the effect of repeated exposure to machining fluid aerosols on the respiratory system. In rats, increases in stored mucus were observed in intrapulmonary airways after only 3 days of exposure. Because these increases were greatest in animals exposed to endotoxin-contaminated machining fluids, it must be stressed again that proper upkeep of machining fluids is essential for reducing adverse respiratory effects.

The pathways leading to lung injury or repair are poorly identified for many occupational agents. As occurs in some workplace settings, adaptation can develop after repeated exposure to inhaled pollutants. Few respiratory changes were observed in guinea pigs exposed for 3 hours/day for 5 days/week for 1 month to used machining fluid aerosols at the current permissible exposure limit. This suggests that adaptation to the acute pulmonary injury associated with exposure to machining fluid aerosol can occur after repeated exposure.

However, this experimentally observed development of tolerance should be interpreted with caution as, in the case of byssinosis, chronic sequelae from long-term exposure to machining fluids could occur.

III. Abstract

This research proposal examined the acute and subchronic pulmonary effects of machining fluid aerosols - an occupational hazard for nearly 1 million workers in this country. Machining fluids/cutting oils are hypoosmolar, alkaline fluids which are frequently contaminated with a variety of microbial agents. The major goals of the proposed studies were to 1) compare the potential of different classes of machining fluids to produce inflammatory and functional changes in the lung; and 2) determine the role of contaminating endotoxin, hypotonicity, and alkalinity in the adverse pulmonary effects associated with acute exposure to machining fluid aerosols. In the first study, guinea pigs were exposed to 3 different machining fluid aerosols. We determined that the type of machining fluid aerosol to which the animals were exposed had a significant effect on pulmonary injury and inflammation (semi-synthetic > soluble >> synthetic). The toxicity of the unused semi-synthetic machining fluid could be reduced by changing its alkaline and hypo-osmolar nature to a pH of 7 and isotonicity. Moreover, the cleanliness of the machining fluid (i.e., the degree of microbial contamination) played a major role in the observed adverse effects. These studies also determined that while inflammation persisted in the lungs of guinea pigs after a month-long exposure to machining fluid aerosols, the pulmonary system could adapt to repeated injury. Thus, the proposed experiments have provided a clearer understanding of the role of microbial contamination and physico-chemical characteristics in the adverse pulmonary effects of inhaled machining fluid aerosols.

IV. Report and Conclusions

Background

Millions of gallons of machining fluids are used in this country for lubrication and cooling applications in machining and cutting operations. Frequently, these fluids are stored in holding facilities with capacities of 10,000 to 25,000 gallons and are delivered under high pressure to machining operations as an aerosol. The potentially exposed populations are enormous: published data indicate that 30,000 workers in Sweden and up to 10 million in the U.S. are exposed to machining fluid aerosols. Despite engineering controls and improved ventilation which limit exposure concentrations to the current permissible exposure limit of 5

mg/m^3, epidemiology and industrial hygiene studies have reported adverse respiratory effects under these conditions.

Inhalation of machining fluid mists has been reported to cause a variety of adverse respiratory effects, including a cross-shift decrease in forced expiratory volume at 1 sec and increases in asthma, bronchitis, and cough. Because these work-related effects appear to occur regardless of the type of machining oil used, it is not clear which machining fluid component is responsible for the development of adverse respiratory effects.

Little is known about the agent(s) responsible for these adverse effects or the potential for chronic injury from repeated exposure to the oil mists. The complexity of the machining fluid aerosols is great: the solutions contain one of a variety of oils (e.g., straight, soluble, or synthetic) as well as biocides and other additives. The solutions are generally alkaline (approximately pH 9), frequently hypoosmolar, and may be contaminated with a wide variety of bacterial and fungal agents. Therefore, evaluating the potential for this complex aerosol to cause acute airway effects is dependent on the individual assessment of the components as well as their possible interactions. Identification of the oil/water component(s) responsible for the adverse health effects associated with machining fluid aerosol exposure is paramount in importance for providing the workers with a healthy work environment. Our research plan examined the relative toxicity of the 3 major classes of machining fluids as well as the contribution of various components/factors of the oils in general. The experiments utilized a guinea pig model which is sensitive to the respiratory effects of inhaled machining fluid aerosols.

SPECIFIC AIM I.A - To determine the relative risk for pulmonary injury from exposure to respirable aerosols of soluble, synthetic, semi-synthetic, and straight machining oils.

and

SPECIFIC AIM I.B - To determine whether endotoxin-contaminated machining fluid aerosols produce greater pulmonary injury than unused machining fluid aerosols.

Single exposures

Methods:

Exposures - Groups of animals were exposed for 3 hours to nebulized water (n = 5) or 3 different types of machining fluids: soluble, semi-synthetic, and synthetic (n = 6 per group). For each machining fluid type, a group of animals was exposed to 2 aerosol concentrations of: 1) an unused machining fluid (unused, neat machining oil diluted to 5% (by volume) in pyrogen-free water (Baxter Laboratories, Deerfield, IL)); 2) a 'clean' machining fluid (a

worksite sample of used machining fluid with minimal microbial contamination); or 3) a 'dirty' machining fluid (a worksite sample of used machining fluid with significant microbial contamination). The target concentrations for each type of machining fluid aerosols were 5 and 50 mg/m^3. Animals were exposed to the lower target concentration only if they responded to 50 mg/m^3. To examine the role of pH and osmolarity on the response to machining fluid aerosols, samples of the unused semi-synthetic machining fluid (50 mg/m^3) and the used dirty semi-synthetic machining fluid (5 mg/m^3) were adjusted to pH 7 (with 1 N sulfuric acid) and/or 300 milliosmoles (with pyrogen-free 0.9 % sodium chloride). The machining fluids were a gift of Cincinnati Milacron (P. Ronald Yust, Cincinnati, OH).

Aerosols were generated with a Babington-type nebulizer (Solosphere, Airlife Inc., Modesto, CA) driven by medical-grade breathing air at 3-14 psi. The output of the nebulizer (4.42 l/min at 9 psi) was diluted with 15 to 43 liters/min air prior to entering the exposure chamber. Airborne machining fluid concentrations were determined gravimetrically by taking samples of the chamber atmosphere in the breathing zone of the animals at 1.07 liters/min during each exposure using 47 mm diameter polyvinyl chloride (0.8 µm, Omega Specialty Instrument Co., Chelmsford, MA) filters. Filters were weighed before and after sampling with a microbalance (Cahn Instruments Inc., Cerritos, CA). Post-sampling filter weights were recorded after the microbalance had stabilized (approximately 2 min) to ensure that low vapor pressure components of the machining fluid, such as water, contributed minimally to the gravimetric analyses. Additional cellulose acetate or glass fiber filter samples were collected, extracted, and analyzed for endotoxin levels using sterile techniques. After sampling, filter media were immediately placed in sterile, pyrogen-free glass containers and stored at 4°C until extracted. After extraction, endotoxin concentrations were quantitated with a *Limulus* amebocyte lysate assay (QC1000, Whittaker Bioproducts, Walkersville, MD) using a spectrophotometric microplate method. The assay results were compared to a standard NBS traceable endotoxin and expressed in terms of endotoxin units (EU) or nanograms (10 EU's was assumed to equal 1 ng). The airborne endotoxin concentrations were expressed in µg/m^3. Particle size measurements for machining fluid aerosols were performed gravimetrically using a Mercer mini-cascade impactor (Intox Products, Albuquerque, NM) with glass substrates. The chemical characteristics and the particle size distributions for the various machining fluids are listed in Tables 1 - 3. Metal analysis of all used machining fluids was done by atomic absorption spectroscopy.

Pulmonary Function. - In each exposure group, specific airway conductance (SGaw) was measured in awake and spontaneously breathing animals at 5 min intervals for 15-20 min prior to exposure and at 60 min intervals during exposure. SGaw was determined in a whole body, constant volume plethysmograph based on the design of Agrawal.

Biochemical Studies - At 24 hr post exposure, guinea pigs were euthanized, tracheostomized, cannulated, and lavaged twice with sterile saline. LDH, protein, total cell counts, and cell differentials were determined in lavage fluid.

Statistical analyses - SGaw values during exposure were normalized to pre-exposure baseline values for each animal. For each exposure concentration, the effect of exposure on SGaw was then analyzed by a two-factor analysis of variance (ANOVA) with independent factors of fluid type and condition (unused, clean, or dirty). When indicated, a Student Newman Keul's test was used to compare normalized SGaw values between all groups. Biochemical and cell count data were also analyzed by a two-factor ANOVA followed by a Student Newman Keul's test when applicable. Levels of $p \leq 0.05$ were accepted as evidence of significant group differences.

Results:

Significant differences in the degree of pulmonary injury and inflammation were observed in guinea pigs exposed to the 3 machining fluid types. Although no significant increases in LDH, protein, or PMNs were found in animals exposed to 50 mg/m^3 of the unused synthetic machining fluid aerosols, both the unused soluble and the semi-synthetic machining fluids produced changes in lavage fluid parameters which were statistically greater than the values of the control animals (Table 4). In the unused soluble and semi-synthetic machining fluids tested at 5 mg/m^3, no significant increases in lavage parameters were noted (Table 4).

The two-factor ANOVA demonstrated that the condition of the machining fluid types significantly affected the outcome of response. A 3 hr exposure to 5 mg/m^3 of the dirty used machining fluid aerosols produced significantly greater pulmonary effects than did the unused or clean used machining fluid aerosols (Table 4). Compared to control animal values, significantly increased levels of LDH activity (Figure 1) and protein were found in the lavage fluid of animals exposed to 5 mg/m^3 of the used dirty synthetic and semi-synthetic machining fluid aerosols. In addition, a significant increase in PMNs occurred in animals exposed to the used dirty synthetic machining fluid aerosols at this exposure level (Figure 2).

Both the pH and osmolarity of the fluid had a significant effect on the response of guinea pigs to unused semi-synthetic machining fluid aerosols. Adjustment of the alkaline and hypotonic fluid to pH 7 and 300 milliosmoles, alone or in combination, significantly reduced the toxicity of a single 3 hr exposure to 50 mg/m^3 of the unused semi-synthetic machining fluid aerosols (Figure 3). Manipulation of the pH and osmolarity, however, did not alter the response of guinea pigs to 10 mg/m^3 of the used dirty semi-synthetic machining fluid (Figure 4).

Machining fluid exposure had no significant effect on SGaw except for a 20% decrease in animals at the end of the 3 hr exposure to either the 5 or 50 mg/m^3 used dirty semi-

synthetic machining fluid aerosols (data not shown). The actual measured machining fluid concentrations for the target concentrations of 5 and 50 mg/m^3 are listed in Table 3. Because no pulmonary effects were observed in the groups of animals exposed to 50 mg/m^3 of either the unused or clean used synthetic machining fluid aerosols, the effect of a lower concentration was not tested. The particle size distribution was similar for all the machining fluid types at each level of exposure (Table 3). The mass median aerodynamic diameter ranged from 0.8 to 1.5 μm and the geometric standard deviation ranged from 1.7 to 2.1. The concentration of metals was similar among the machining fluids with the exception of a greater level of Cu and Zn in the unused semi-synthetic machining fluid.

Conclusions:

As a first step in determining the contribution of the chemical constituents to the toxicity of machining fluid aerosols, the present study examined the adverse functional, biochemical, and cellular effects of the 3 major fluid types currently used in machining operations. The study clearly demonstrated that fluid type had a significant impact on outcome. Following exposure to the unused machining fluids at the high concentration of 50 mg/m^3, only the semi-synthetic fluid produced significant increases in two of the three parameters of lung injury and inflammation examined. Exposure to the high concentration of the unused soluble oil produced a significant increase only in LDH activity, while no significant changes were observed in the animals exposed to 50 mg/m^3 of the unused synthetic machining fluid aerosols. Although these findings may not apply across the board to the many and continually changing formulations of the three major machining fluid types, they do point out that differences in toxicity do exist among the machining fluids and should be considered in the use and future design of machining fluids.

The present study also determined that for each of the three different machining fluid types, the used dirty (i.e., poorly maintained) machining fluids were more toxic than the used clean (i.e., well-maintained) or unused machining fluids. This finding confirms earlier work and suggests that changes in the chemical constituents or microbial contamination of machining fluid contribute strongly to the production of lung injury and inflammation in this guinea pig model. As shown in Table 1, there were no major differences in the metal content, pH, and osmolarity of the used machining fluids and, thus, these factors likely played little role in the observed inter-fluid differences in toxicity. Extensive chemical analyses of the unused and used machining fluids were not performed and would be necessary to elucidate the possible contribution of other factors, such as the organic components, to the observed adverse respiratory effects. Importantly, the increases in adverse effects produced by the used, dirty machining fluid aerosols were associated with the bacterial titer of the fluids, as well as the presence of endotoxin in the aerosols. Thus, as suggested by previous work in this lab, it is

likely that contaminating endotoxin contributes to the adverse effects of poorly-maintained machining fluids.

The alkaline pH and the hypotonicity of the machining fluids appear to play a role in the adverse effects associated with inhalation of machining fluid aerosols. This study demonstrated that adjusting the pH and osmolarity to 7.0 and 300 milliosmoles, respectively, markedly inhibited the effects of exposure to 50 mg/m^3 of the unused semi-synthetic machining fluid. The relative contribution of these physico-chemical factors, however, was apparently less than that of the contribution of the changes in machining fluids that occur during use and storage at the worksite. This was demonstrated by the lack of inhibition of observable adverse effects in animals exposed to 5 mg/m^3 of the pH- and osmolarity-adjusted, used, dirty, semi-synthetic machining fluid aerosols. In conclusion, animal studies have the capability to provide important information regarding the toxicity of complex mixtures such as machining fluid aerosols. This study demonstrated inherent differences in the toxicity of the 3 major machining fluids used in machining operations today. Thus, end users may consider the potential toxicity, in addition to the functionality, of the machining fluid types in the choice of cutting and lubricating fluids for machining operations. Alternatively, machining fluid manufacturers may be able to adjust the chemical components to reduce the respiratory toxicity of machining fluids. The present study also presented evidence that a single 3 hr exposure to 5 mg/m^3 (the current 8 hour permissible exposure level) of unused machining fluids does not produce any observable functional, biochemical, or cellular adverse effects in an animal model. Thus, workplace exposure to well-maintained and non-contaminated machining fluid aerosols may be without acute adverse effects. During the routine use and storage of tens of thousands of gallons of machining fluids, however, bacterial and fungal contamination does occur. The present study has demonstrated that microbial contaminants, pH, and osmolarity may each contribute to the acute, adverse effects reported for machining fluid-exposed workers.

Repeated exposures

Methods:

After completion of the single exposure studies, additional animals were exposed to nebulized water (the vehicle), 1 or 5 mg/m^3 used semi-synthetic machining fluid, or 5 mg/m^3 unused semi-synthetic machining fluid aerosols for 3 hr/day, 5 days/week for 4 weeks. The semi-synthetic machining fluid and the concentrations used in the 30 day study were chosen based upon results of the single exposure study. For each of the 4 exposure conditions, 10 guinea pigs were exposed in 1.6 m^3 stainless steel and glass Laskin chambers. Machining fluid aerosols were generated and monitored as described above. Six animals were euthanized immediately after the final day of exposure and examined for changes in lavage parameters

and morphology. The remaining animals were used for monitoring changes in SGaw and airway responsiveness to acetylcholine throughout the 4 week study.

Results:

No significant changes in lavage parameters, stored mucosubstances, SGaw, or airway responsiveness were observed except for a 6-fold increase in PMNs in the lavage fluid recovered from animals exposed to 5 mg/m^3 used semi-synthetic machining fluid aerosols.

Conclusions:

These results suggest that adaptation to the adverse pulmonary effects observed after a single exposure, can develop upon repeated exposure to machining fluid aerosols. This experimentally observed development of tolerance must be interpreted with caution as the sequelae from long-term exposure to machining fluids are still unknown. As demonstrated in this study, inflammatory changes (i.e., increases in PMNs) did occur in the lungs of guinea pigs who developed tolerance to other adverse effects of machining fluid aerosols.

SPECIFIC AIM I.C - To determine whether inhaled endotoxin-contaminated machining fluid aerosols alter mucus cell populations in the airways and thereby interfere with non-specific lung defense.

Methods:

In collaboration with Dr. Jack Harkema (Michigan State University), this laboratory examined in the rat whether repeated exposure to occupationally relevant concentrations of used and unused machining fluids alters the amount of stored mucus in the respiratory tract. The rat was used in our model of mucous cell metaplasia instead of the guinea pig because of the significant amount of data (produced in Dr. Harkema's and Dr. Gordon's labs) that the Fischer 344 rat is more sensitive to the mucus inducing effects of inhaled pollutants. Rats (n=6 per group) were exposed to aerosols of pyrogen-free water or 1 and 10 mg/m^3 used machining fluid for 3 hours/day for 3 days and sacrificed at 24 hours after the final exposure. Additional animals were exposed to 10 mg/m^3 unused machining fluid aerosols to compare the response of animals to aerosols of used versus unused machining fluid. Quantitative histochemistry of mucus in airway epithelium was examined at 2 levels of the respiratory tract: the nasal septum and intrapulmonary airway (generation 5). The effect of machining fluid exposure on stored mucosubstances in the nasal septum and axial intrapulmonary airway and on biochemical and cellular parameters in lavage fluid was analyzed by a one way analysis of variance. Post-hoc analyses of group differences were performed with a two-way Dunnett's *t* test to compare control group values with machining fluid groups values.

Results:

Exposure to used (endotoxin-contaminated) machining fluid produced a dose-dependent increase in stored mucus in the respiratory tract (Figures 2 and 3 in the manuscript titled "Mucous Cell Metaplasia in the Airways of Rats Exposed to Machining Fluids", Fund Appl Toxicol 28:274-282, 1995 - see Appendix). Mucus in the intrapulmonary airways and nasal septum was significantly increased in animals exposed to 10 mg/m^3 used machining fluid aerosols, while no significant changes were observed after exposure to 1 mg/m^3. The changes in stored mucosubstances were accompanied by significant increase in neutrophils and total cells in bronchoalveolar lavage fluid. Interestingly, exposure to 10 mg/m^3 unused machining fluid produced a significant increase in stored mucosubstances in the nasal septum, but not the intrapulmonary airway.

Conclusions:

The 3 day exposure protocol demonstrated that machining fluid aerosols produce a rapid induction of stored mucosubstances in the respiratory tract of the rat. The similarity in the time course of induction by both inhaled endotoxin (previous work) and machining fluids suggests that endotoxin plays a role in the induction of intrapulmonary stored mucosubstances by used machining fluids. However, it is clear from the results in the nasal septum that an endogenous component(s) of the soluble machining fluid used in this study has the ability to induce a change in stored mucosubstances in the absence of endotoxin contamination. This study has also demonstrated that the induction of goblet cell metaplasia in rat airways is as sensitive a marker of the adverse effects of repeated exposure to machining fluid aerosols as is the influx of PMNs, a general marker of inflammation. Finally, because the current time-weighted average (8 hr) permissible exposure limit for general machining fluids is 5.0 mg/m^3 in most countries, the induction of goblet cell metaplasia in rats exposed to 10 mg/m^3 machining fluid for 3 hr/day for 3 days is relevant to the assessment of human health risks and to the understanding of occupationally-induced chronic bronchitis. Importantly, the present findings suggest an *in vivo* model for the study and identification of machining fluid components responsible for the development of sputum production and chronic bronchitis in workers chronically exposed to these bio-aerosols.

V. Acknowledgments

The investigators would like to thank P. Ronald Yust (Cincinnati Milacron, Cincinnati, OH) for assistance in obtaining and characterizing the machining fluids used in these studies and Dr. Jack Harkema for guidance in the morphometric analysis of stored mucosubstances. We would also like to acknowledge the superb technical work of Karen Galdanes.

VI. Publications

Gordon T, Harkema JR: Mucous Cell Metaplasia in the Airways of Rats Exposed to Machining Fluids. Fundamental Applied Toxicology 28:274-282, 1995

Gordon T, Galdanes K: Factors Contributing to the Adverse Respiratory Effects of Machining Fluid Aerosols in Guinea Pigs (submitted for publication)

Gordon T: The Development of Tolerance in Guinea Pigs Exposed to Machining Fluid Aerosols for 30 Days (planned for publication)

White E, Howell J, Gordon T, et al: An Assay for Measuring Endotoxin in Machining Fluids (planned for publication as an ASTM protocol)

TABLE 1

Chemical and microbial characteristics

FLUID TYPE	CLASS	METAL CONC (ppm)				pH	OSMOLARITY	BACTERIAL TITER	MOLD TITER
		Cd	Cu	Pb	Zn		milliosmoles		
SOLUBLE	UNUSED	0.0	0.0	0.0	0.14	7.0	69	ND	ND
SOLUBLE	CLEAN	0.16	0.62	3.0	0.07	8.8	109	6.5E+04	1.0E+03
SOLUBLE	DIRTY	0.16	0.74	2.3	0.92	7.8	94	1.0E+07	BDL
SEMI-SYNTHETIC	UNUSED	0.0	4.0	0.0	7.4	9.0	81	ND	0.0E+00
SEMI-SYNTHETIC	CLEAN	0.3	0.67	0.36	0.96	8.8	111	1.0E+03	0.0E+00
SEMI-SYNTHETIC	DIRTY	0.0	0.0	0.11	0.0	9.0	142	1.0E+06	0.0E+00
SYNTHETIC	UNUSED	0.0	0.0	0.0	0.0	9.5	147	ND	ND
SYNTHETIC	CLEAN	0.0	0.21	0.0	0.24	9	192	1.0E+06	0.0E+00
SYNTHETIC	DIRTY	0.0	0.46	0.0	0.52	8.5	149	1.0E+06	0.0E+00

ND = not determined

Table 2 - Chemical Constituents of Machining Fluids

MACHINING FLUID	COMPONENTS	MAX %
SOLUBLE	MINERAL OIL	50
	ETHANOLAMINE	10
	NONYLPHENOXY-POLYETHOXYETHANOL	10
	TRIETHANOLAMINE	10
SEMI-SYNTHETIC	MINERAL OIL	30
	ETHANOLAMINE	10
	NONYLPHENOXY-POLYETHOXYETHANOL	10
	TRIETHANOLAMINE	10
	ORGANIC ACID	10
SYNTHETIC	ETHANOLAMINE	10
	CAPRYLIC ACID	10
	TRIETHANOLAMINE	10
	ISONONANOIC ACID	10

Table 3

EXPOSURE ATMOSPHERE PARAMETERS

EXPOSURE	n	OIL CONC (mg/m^3)	E NDO CONC ($\mu g/m^3$)	MMAD (μm)	GSD
WATER	5	NA	BDL	NA	
SOLUBLE-UNUSED-5 mg/m^3	6	4.5	BDL	1.0	1.80
SOLUBLE-UNUSED-50 mg/m^3	6	52.8	0.05	1.2	1.93
SOLUBLE-CLEAN-5 mg/m^3	6	5.2	BDL	1.0	1.72
SOLUBLE-CLEAN-50 mg/m^3	6	52.0	0.01	1.3	1.85
SOLUBLE-DIRTY-5 mg/m^3	6	5.1	0.04	1.0	1.75
SOLUBLE-DIRTY-50 mg/m^3	6	49.1	0.45	1.4	1.76
SEMI-SYNTHETIC-UNUSED-5 mg/m^3	6	6.3	BDL	0.8	1.71
SEMI-SYNTHETIC-UNUSED-50 mg/m^3	6	51.9	0.01	0.8	1.71
SEMI-SYNTHETIC-CLEAN-5 mg/m^3	6	5.2	BDL	1.1	1.69
SEMI-SYNTHETIC-CLEAN-50 mg/m^3	6	45.6	BDL	1.5	1.83
SEMI-SYNTHETIC-DIRTY-5 mg/m^3	6	5.6	0.13	0.9	1.75
SEMI-SYNTHETIC-DIRTY-50 mg/m^3	6	47.5	0.55	1.2	2.00
SYNTHETIC-UNUSED-5 mg/m^3	NT	NT	NT	NT	NT
SYNTHETIC-UNUSED-50 mg/m^3	6	53.5	0.13	1.1	1.95
SYNTHETIC-CLEAN-5 mg/m^3	NT	NT	NT	NT	NT
SYNTHETIC-CLEAN-50 mg/m^3	6	49.3	0.01	1.2	1.79
SYNTHETIC-DIRTY-5 mg/m^3	6	7.2	0.27	0.8	2.04
SYNTHETIC-DIRTY-50 mg/m^3	6	48.9	1.92	1.1	2.09

NA = Not applicable.
NT = Not tested because no adverse effects were observed after exposure to 50 mg/m^3 of this machining fluid.
BDL = Below detection limit.
MMAD = Mass median aerodynamic diameter
GSD = Geometric standard deviation

Table 4

EFFECT OF MACHINING FLUID EXPOSURE ON BALF AND SGaw PARAMETERS[a]

EXPOSURE	50 mg/m^3 LDH	50 mg/m^3 PROTEIN	50 mg/m^3 PMNS	5 mg/m^3 LDH	5 mg/m^3 PROTEIN	5 mg/m^3 PMNS
SOLUBLE-UNUSED	+	-	-	-	-	-
SOLUBLE-CLEAN	+	+	+	-	-	-
SOLUBLE-DIRTY	+	+	+	-	-	-
SEMI-SYNTHETIC-UNUSED	+	+	-	-	-	-
SEMI-SYNTHETIC-CLEAN	+	+	+	-	-	-
SEMI-SYNTHETIC-DIRTY	+	+	+	+	-	-
SYNTHETIC-UNUSED	-	-	-	NT	NT	NT
SYNTHETIC-CLEAN	-	-	-	NT	NT	NT
SYNTHETIC-DIRTY	+	+	+	+	-	+

[a] + = Statistically different ($p < 0.05$) from the control (water) group values as determined by a Dunnett's two-tailed t test.
- = Not statistically different from the control group values.

NT = Not tested because no significant changes occurred after exposure to a higher concentration.

Figure 1 - The Effect of Used Dirty Machining Fluid Aerosols on LDH Activity in Lavage Fluid

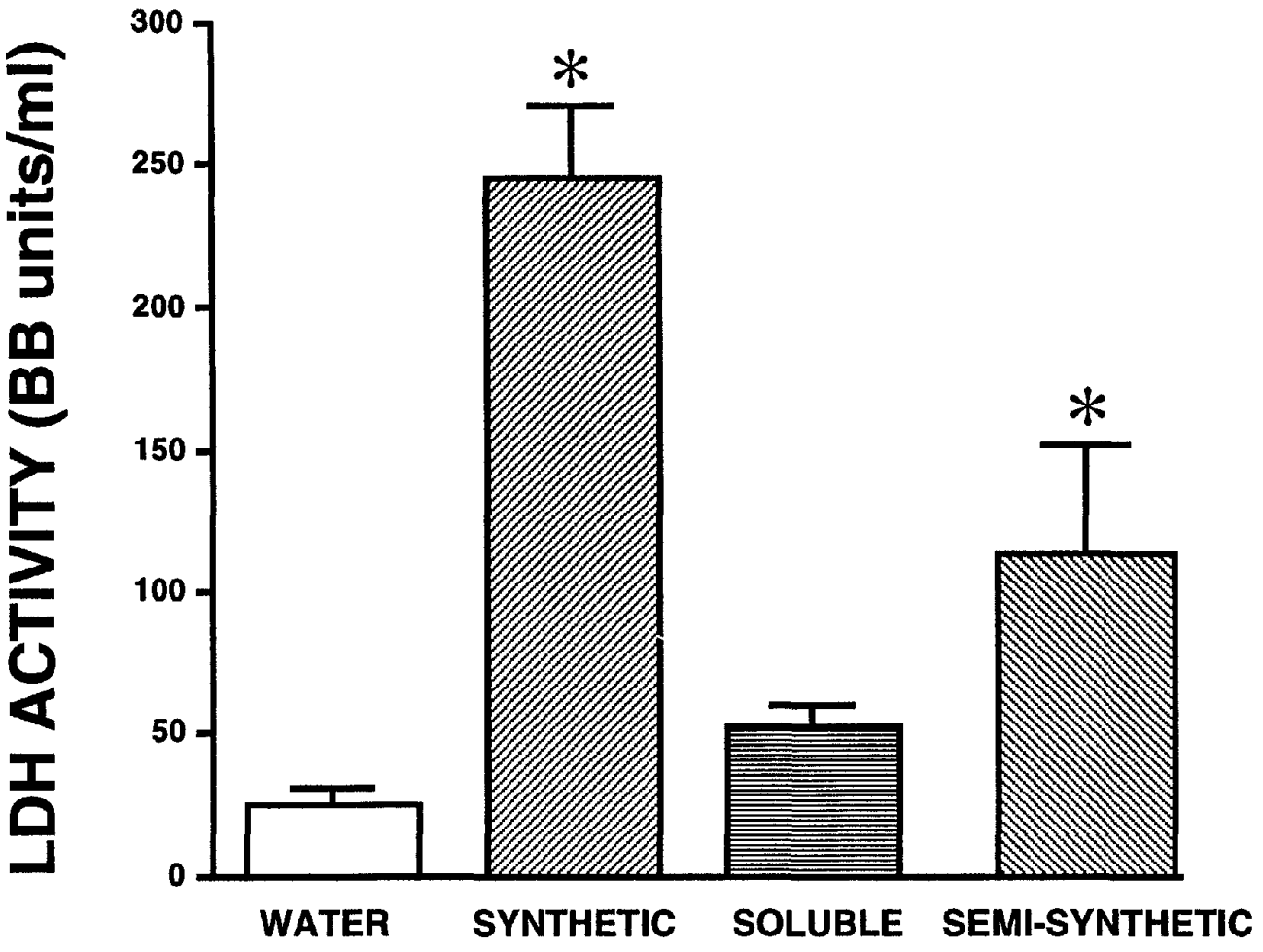

Figure 2 - The Effect of Used Dirty Machining Fluid Aerosols on Total PMNs in Lavage Fluid

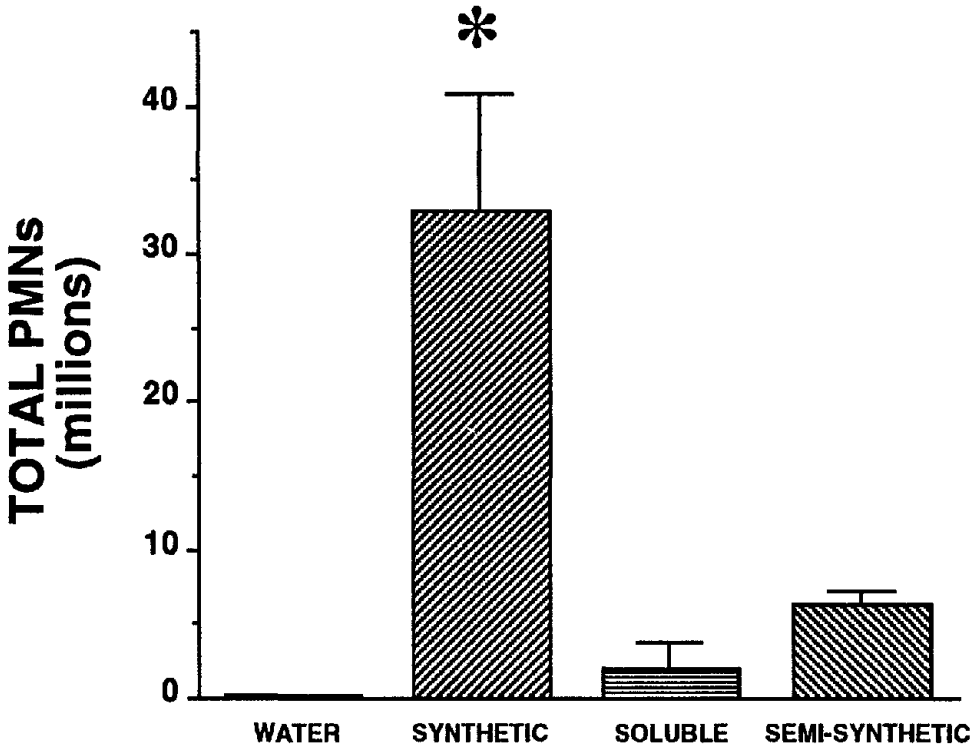

Figure 3 - The Effect of Changing pH and Osmolarity on the Response to 50 mg/m3 Unused Semi-Synthetic Machining Fluid Aerosols

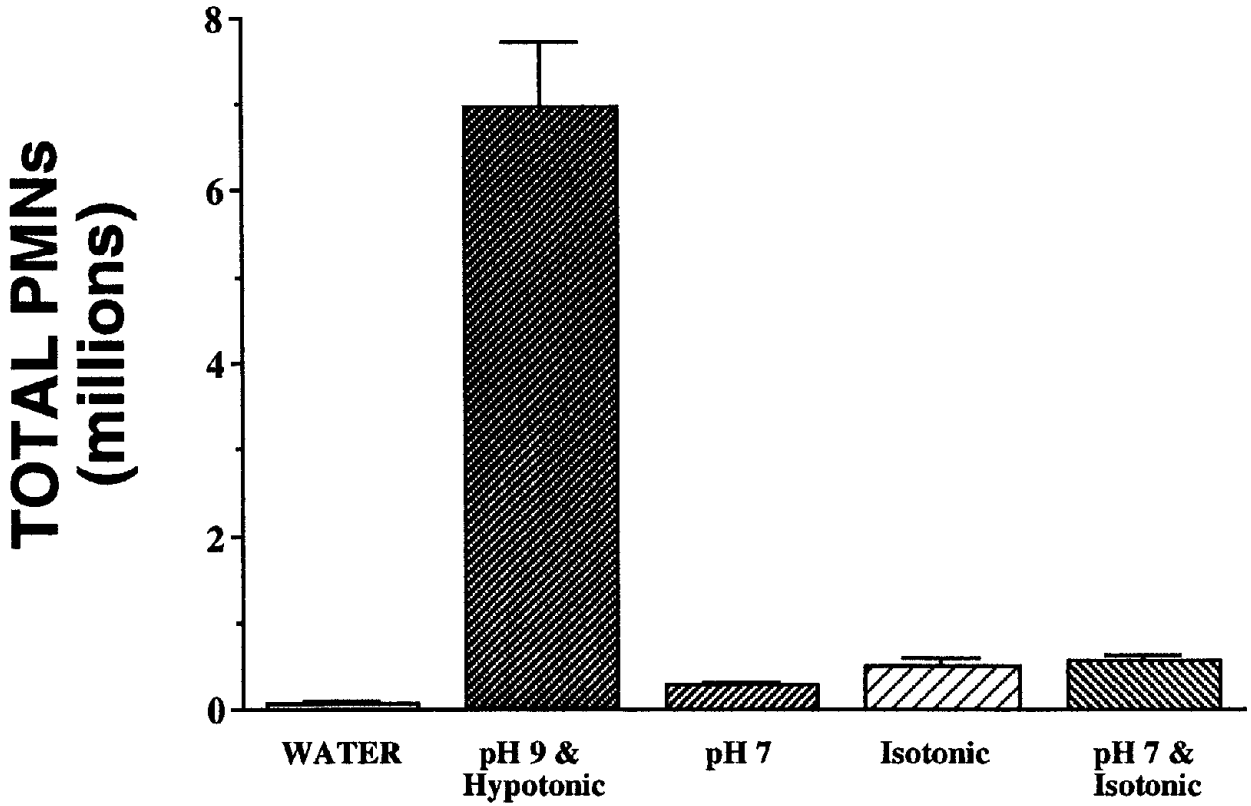

Figure 4 - The Effect of Changing pH and Osmolarity on the Response to 10 mg/m3 Used Dirty Machining Fluid Aerosols