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METAL FUME FEVER

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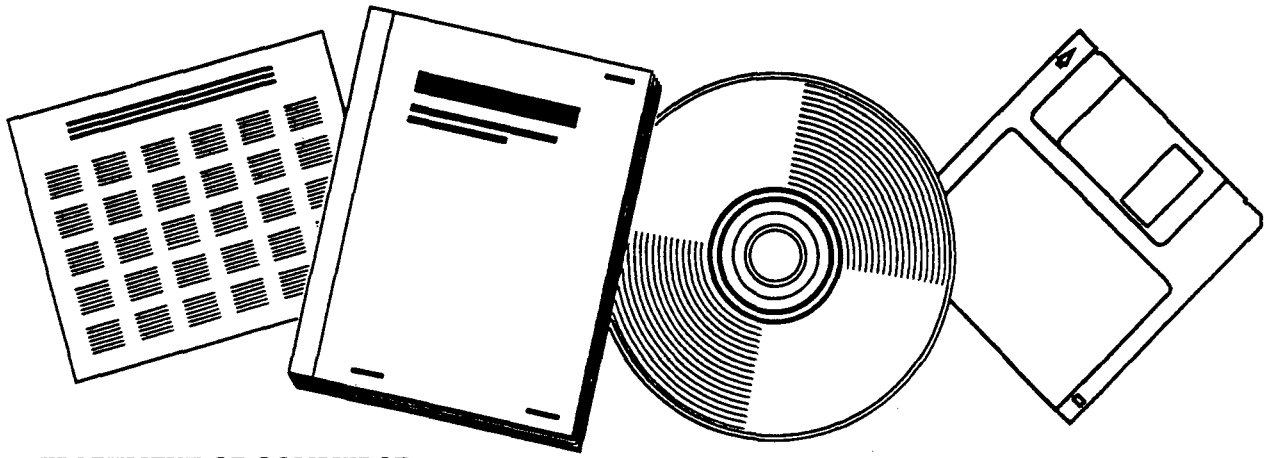

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Final Performance Report

"METAL FUME FEVER"
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16. Abstract (Limit: 200 words) The acute response to zinc-oxide (1314132) fumes in naive human subjects and galvanized sheet metal workers was investigated. Of 12 naive subjects, ten experienced a mild fever after a 2 hour exposure to zinc-oxide fume at 5mg/m3 (the OSHA permissible exposure limit) or 2.5mg/m3. Metal fume fever symptoms were significantly increased 6 and 9 hours after a 5mg/m3 exposure. The most common symptoms were fatigue, muscle ache, and cough. Elevated white blood cell counts were also seen. Those who experienced mild symptoms and fever developed tolerance of these effects with repeated exposures on subsequent days. Sheet metal workers with ongoing low level exposure showed tolerance to exposure at 5mg/m3. However, zinc-oxide exposure in these individuals induced elevated levels of interleukin-6 in the blood. The findings demonstrated that mild symptoms and fever can occur in previously unexposed men and women several hours after they breathe ultrafine zinc-oxide fumes for only 2 hours at the 5.0mg/m3 level. This occurred in a high proportion of healthy and normal individuals.

17. Document Analysis a. Descriptors

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I. SIGNIFICANT FINDINGS

A. The common occupational illness metal fume fever was reproduced in the laboratory using pure, ultrafine zinc oxide fume under blinded, controlled human inhalational exposure conditions. The illness produced included the cardinal characteristics of fever, constitutional symptoms, and elevated serum white blood cell count. Cough, myalgia, and fatigue were the most prominent symptoms.

B. Under these controlled laboratory conditions, mild metal fume fever occurred in the majority of previously unexposed healthy male and female subjects after a single exposure for two hours at the current OSHA PEL of 5 mg/m_3 zinc oxide fume. Some of the characteristic findings also occurred after a comparable exposure at 2.5 mg/m^3, but the effects at this dose were not statistically significant.

C. The pro-inflammatory cytokine interleukin-6 (IL-6) was elevated in blood with peak levels at three to six hours after exposure to zinc oxide fume. Peak IL-6 levels preceded the peak fever response. Blood tumor necrosis factor (TNF) was not elevated.

D. Sheet metal workers, chronically exposed to zinc oxide fume at work through use of galvanized metal thermal cutting equipment, demonstrated tolerance to acute challenge with zinc oxide fume, as seen by lack symptoms and temperature response, *even though they have not previously suffered an acute episode of metal fume fever*. However, sheet metal workers demonstrated elevated levels of the pro-inflammatory cytokine IL-6 in blood.

E. In previously unexposed subjects who responded to an initial two hour zinc oxide fume challenge at 5 mg/m^3, tolerance (as measured by total symptom score and maximal fever) was induced with two subsequent identical challenges at the same concentration on separate days. Clinical tolerance was also associated with a reduction in blood levels of the pro-inflammatory cytokine IL-6, suggesting an important mechanistic role for IL-6 in the clinical illness of metal fume fever.

F. Metallothionein mRNA induction was not found in peripheral blood lymphocytes of acutely exposed naive individuals, nor in nasal epithelial brushings of acutely exposed naive individuals. Based on the findings from our laboratory of metallothionein mRNA induction in homogenized lung tissue from animals similarly exposed, we speculate that metallothionein mRNA induction would be seen in lung tissue of exposed individuals.

II. USEFULNESS OF FINDINGS.

Metal fume fever is a common, self-limited febrile illness caused by inhalation of freshly generated metal oxide fumes. Stella Horoshak, herself a brass foundry worker, described this episode of metal fume fever in her husband: "One night my husband was shaking. I didn't know what happened. I said 'I'm going to call a doctor.' He said, 'No. Just get me a lot of blankets.' Their body gets to a certain temperature, and they start shaking. Like a malaria. He told me after about four hours he was all right. In the thirty three years I was married it happened four times to him."[1]

It is estimated that 1500 to 2000 cases of this occupational illness occur in the United States each year. Previous clinical and research experience indicate that tolerance to the fever and symptoms is rapidly induced by a single acute illness, so that an individual who promptly returns to the same workplace exposure will not have a recurrence of the illness.

This research demonstrated that mild symptoms and fever can occur in previously unexposed men and women several hours after they breathe ultrafine zinc oxide fume for only two hours at the current OSHA Permissible Exposure Limit of 5.0 mg/m^3 zinc oxide. Individuals need not be particularly sensitive for this to occur; rather it may occur in a high proportion of healthy normal individuals. Subjects who experienced mild symptoms and fever developed tolerance to these effects with repeat exposures at this level on subsequent days. Sheet metal workers with ongoing low-level exposure to zinc oxide fume (too low to cause symptoms) appeared to have already developed tolerance to the symptoms and fever with identical laboratory challenge to zinc oxide at the OSHA PEL. In these individuals, however, zinc oxide exposure induces elevated levels of the pro-inflammatory cytokine interleukin-6 in blood as well as in the blood.

Previously unexposed individuals who developed tolerance (by symptoms and/or temperature response) to three exposures of two hours at the OSHA PEL also showed a reduction in the blood levels of interleukin-6, indicating that this pro-inflammatory cytokine plays an important role in the clinical illness of metal fume fever.

Some of these results have already been reported in abstract form at the largest international meeting of lung physicians, to alert them to the occurrence of mild metal fume fever after inhalation exposures previously considered to be protective. Other results will be presented both in scientific meetings and in scientific and medical journals.

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[1] *Brass Valley*. The Story of Working People's Lives and Struggles in an American Industrial Region. Compiled and edited by Jeremy Brecher, Jerry Lombardi, and Jan Stackhouse. Philadelphia, Temple University Press.

III. ABSTRACT

Metal fume fever is a common occupational illness caused by inhalation of freshly generated zinc oxide fume. It is characterized by fever, systemic symptoms, and mild lung inflammation, with onset several hours after the acute exposure. This illness has historically been common among brass foundry workers and welders working on galvanized (zinc-coated) sheet metal. We investigated the dose response relationships of zinc oxide fume inhalation to metal fume fever in naive and chronically exposed individuals, and examined the mechanisms of the illness and the development of tolerance with serial exposures. Using a laboratory electric furnace-generated ultrafine (0.07 micron mass median aerodynamic diameter) zinc oxide particulate at and below the current OSHA PEL of 5.0 mg/m^3, we tested the responses of a group of healthy normal never-exposed individuals and chronically low-level exposed sheet metal workers, looking at the responses of symptoms, fever, blood cell counts and cytokines, and lung lining fluid (broncho-alveolar lavage fluid), cell counts and cytokines. In a blinded protocol, previously unexposed healthy men and women demonstrated the cardinal features of metal fume fever including mild symptoms, fever, and elevated white blood cell count several hours after exposure. Sheet metal workers using thermal cutting tools in fabrication shops were found to be chronically exposed to zinc oxide. In contrast with the naive subjects, at the same maximal exposure in the laboratory, the chronically exposed sheet metal workers had no significant increase in symptoms or oral temperature, but demonstrated an elevation in blood interleukin-6 after challenge at 5 mg/m^3. Naive subjects who responded to a single two hour challenge at 5 mg/m^3 demonstrated tolerance (in symptoms, temperature response, and blood interleukin-6) in a series of three daily challenges. Induction of metallothionein in the lungs of experimental animals may explain the tolerance to inhaled zinc oxide, but an assay for induction of metallothionein messenger RNA (mRNA) in nasal epithelial cell brushings of acutely exposed subjects before and one day after exposure did not show a significant change. We conclude that zinc oxide ultrafine particle inhalation produces metal fume fever with a striking, rapid tolerance on serial exposure, as predicted by the workplace experience. In addition, low-level zinc oxide exposure without symptoms also induces tolerance to a subsequent higher-level challenge. Mild symptoms, fever, and elevation of interleukin-6 are produced in a high proportion of healthy normal men and women after only two hours exposure at the current OSHA PEL of 5 mg/m$_3$ zinc oxide.

IV. REPORT AND CONCLUSIONS (Numbered by Specific Aims)

Specific Aim I. Characterization and mechanism of the acute response to zinc oxide fumes in human subjects.

A. To examine the type and severity of the symptomatic and febrile response of naive human subjects exposed to zinc oxide fume at the current OSHA PEL of 5 mg/m^3 and to seek a "no or minimal effect" level of exposure for acute symptoms and fever.

Exposure. The zinc oxide generating furnace and exposure system produced spherical primary particles with size approximately 0.008 micrometer (80 Angstrom) which aggregate in air before deposition in the human respiratory system. Secondary chain-aggregate particles were measured with a differential mobility analyzer (TSI, model 3021, St. Paul, MN) and found to have a count median diameter of 0.06 micron (1.8 GSD). Using the Hatch-Choate conversion equation, the mass median aerodynamic diameter of these particles would be predicted to be 0.17 micron (1.5 GSD). For purposes of comparison, fresh welding fume measured by other investigators has been reported in the range of 0.06 to 0.52 micron MMAD.

During each two hour exposure period, zinc oxide was measured from a port in the upstream inhalation tubing of the exposure system at 20 minute intervals by collecting zinc oxide on a Teflon filter and determining concentration on a Cahn gravimetric balance. The means of the 6 measurements for each exposure session of all 12 subjects were taken. For the 2.5 mg/m^3 target exposures, the mean exposure was 2.52 mg, (SD .268), and for the 5.0 mg target exposures the mean was 4.89 mg/m^3, (SD .401). For the 2.5 mg/m^3 target concentration, the range of individual measurements was 0.59 to 5.3 mg/m^3, and for the 5.0 mg^3 measurements the range was 0.91 to 8.6 mg/m^3. Exposures were single-blinded and controlled with exposure to furnace gas without added zinc. Under exposure and control conditions, the vehicle gas carrying zinc oxide fume was composed of approximately 97% air and 3.3% argon (a gas which is inert with respect to the lungs).

Figure 1 shows mean air concentrations of zinc oxide at the six time points over the two hour exposures for the twelve naive subjects in the dose-response study for both the 5.0 and 2.5 mg target air concentrations.

Under these conditions and assuming a minute ventilation for resting adult subjects of approximately 6 liters per minute, the total exposure to the nose and lips at the 5.0 mg target dose was approximately 3.6 mg zinc oxide fume over the two hours of exposure. The deposition fraction of ultrafine zinc oxide particulate in man is not known, and has found to be from 5 to 40% in experimental animals. If we assume a deposition fraction of 20% in our subjects, then the dose of ZnO deposited in the total respiratory system would be estimated at 0.72 mg per two hour exposure.

Response. Most of the previously unexposed normal subjects demonstrated mild findings of metal fume fever under these controlled experimental conditions. Among the 12 subjects, 10 experienced a mild fever (defined as a rise in temperature from pre-exposure baseline of $\geq$ 1 degree F.), while 3 experienced a similar rise in temperature after control gas, thus indicating an abnormal fever response in 7 of 12 subjects. Mean temperatures were significantly increased over pre-exposure control for both the 5.0 and 2.5 mg exposures (Figure 2) and by 24 hours after exposure had declined toward control levels. Four subjects recorded temperatures greater than 100 degrees F, and the peak temperature recorded was 100.9 degree F. The mean maximal rise in temperature over baseline was 1.35 degree F. for the 5 mg exposure, and 1.23 degree F. for the 2.5 mg exposure.

Typical metal fume fever symptoms as recorded on a Borg scale at 3 hour intervals were significantly increased in aggregate at 6 and 9 hours after the 8 a.m. until 10 a.m. exposure for the 5.0 mg exposure but not for the 2.5 mg exposure. A negative control symptom (itching) included in the symptom questionnaire was not reported by any subject under any of the exposure circumstances.

The symptoms most frequently noted were fatigue, muscle ache, and cough, and the mean score for each of these symptoms was significantly elevated at 9 hours after exposure. Subjects rated most symptoms in the slight to mild range with only 4 subjects grading any symptom as moderate, and none as severe. No significant differences in symptom reporting were found between women and men.

Conclusion. In an animal toxicologic study preliminary to these human studies, Lam et al (AIHAJ 1988; 49:333-341) found reversible pulmonary function changes in guinea pigs exposed three hours daily for five days to 7 mg/m^3 zinc oxide fume, using the same fume generating furnace system used in these studies. One the basis of these studies, the authors suggested that the PEL of 5 mg/m^3 may be inadequate to protect against respiratory effects. Our human exposure studies were designed to examine that question looking at typical fever and symptom responses, and within the exposure parameters specified (ultrafine zinc oxide particulate) we confirm that naive subjects do experience metal fume fever at the PEL. At one half the PEL (2.5 mg/m^3) the response in this group was less or not measurable, indicating that this level is close to a no or minimal effects level.

B. To determine whether specific fever-producing and pro-inflammatory cytokines are released following zinc oxide exposure and, if so, whether their presence correlates with the occurrence of the clinical metal fume fever syndrome.

Peripheral venous blood was drawn from subjects' arms at 0, 3, 6, and 24 hours after exposure; measurements of the pro-inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor (TNF) were performed using highly sensitive enzyme linked immunosorbent assays (ELISA) and a microtiter plate reader. Interleukin 6 (Figure 5) but not TNF was significantly elevated for both 2.5 and 5.0 mg exposures, with peak levels of approximately 4 picograms per liter at 6 hours after exposure (as compared with mean baseline levels of approximately 1 picogram per liter). IL-6 levels were highest at 6 hours after exposure (no 9 or 12 hour measurements), while symptoms were greatest at 9 hours, and temperature greatest at 10-11 hours. By 24 hours after exposure IL-6 levels had returned almost to baseline levels. These findings are consistent with the original hypothesis that interleukin-6 is active as a pro-inflammatory cytokine in metal fume fever, and may be causally involved in the chain of events

between deposition of zinc oxide in lung and the occurrence of the typical symptoms and fever.

Total white blood cell count (WBC) was drawn at only two time points, immediately before and 12 hours after exposure (Figure 6). As had been seen previously in early studies with higher exposure, WBC is mildly increased 24 hours after zinc oxide exposure (Figure 6). The rapid time course of the initial rise seen in the previous studies, as well as the relatively modest rise in white count is more consistent with a "de-margination" or detachment of white cells vascular endothelium rather than with a bone marrow response to the inhaled substance. Interleukin-6 can activate granulocyte colony stimulating factor (GCSF) which in turn can activate the marginated pool of white cells, as well as affecting bone marrow production of white cells.

II. Adaptive response of human subjects to repeated exposure to zinc oxide fumes.

A. To compare acute clinical response of naive individuals and galvanized metal workers to zinc oxide fumes, we will determine whether chronic exposure of galvanized sheet metal workers to metal fumes at the workplace confers relative tolerance to zinc oxide fumes at the current OSHA PEL of 5 mg/m^3.

Under identical laboratory conditions as the naive subjects reported above, skilled sheet metal fabrication shop workers with chronic exposure to low levels of zinc oxide fume *but not previous episodes of metal fume fever* were found to have much less symptomatic and fever response to zinc oxide at the OSHA PEL.

Chronic ZnO exposure. Sheet metal workers were recruited based on employment that included regularly using thermal cutting tools (e.g. laser cutters) indoors in sheet metal fabrication shops. Appropriately exposed workers were recruited with the generous assistance of individual members and officers of Sheet Metal Workers International Association Local #40 and particularly Mr. James Neary and Mr. Guy DiMaggio. Shops were first visited by the research industrial hygienist Judith Sparer CIH, and air monitoring conducted at graduated distances from the process during thermal cutting operations to ascertain presence of zinc oxide in air and the magnitude of exposure. The floor plan of one of two shops visited (labelled Shop A) is shown in Figure 9, and corresponding air monitoring levels of zinc oxide in Figure 10. The numbers of locations specified in the diagram of Shop A (Figure 9) correspond with individual air monitoring levels in units of mg/m^3 zinc oxide in air, specified in figure 10.

To further verify ongoing zinc oxide exposure in the sheet metal workers, 24 hour urine samples for zinc (the usual assay for zinc oxide exposure) were collected from sheet metal workers one to two days prior to inhalational challenge testing in the laboratory. Results from seven sheet metal workers are shown in Figure 11. Normal zinc excretion from dietary sources and metabolic breakdown of zinc in tissue ranges from 150-1250 microgram per 24 hours (in the reference population of the Smith Kline commercial laboratory which performed these analyses). The mean value for sheet metal workers in the study was 1014 micrograms per sample, within the normal range but in the upper 10th per centile of this normal range. One sample exceeded the upper range of normal. The mass of zinc excreted by these subjects indicates that their total zinc intake (both by ingestion and inhalation) exceeds that of the

average healthy population, but that it is only very mildly elevated.

Laboratory Exposure. The nine chronically exposed male sheet metal workers underwent blinded laboratory exposure to zinc oxide fume at 0 and 5.0 mg/m^3 for two hours in the morning in the same protocol as described above for naive subjects in the dose response study, and their responses were compared to naive subjects.

Responses. The chronically exposed sheet metal workers were on average older than the normal subjects, and were all males, but otherwise not clinically distinguishable from the naive subjects. These chronically exposed workers had not previously had a symptomatic episode of metal fume fever. Mean temperature responses and total symptom responses (Figure 12) were less than those of naive subjects, and were not statistically different from temperatures and symptoms recorded on control exposure days.

These results show a new finding, that chronic low level, asymptomatic exposure to zinc oxide produces a state of adaptation or tolerance to the symptoms of metal fume fever.

By contrast, interleukin-6 levels in the blood of sheet metal workers were higher after ZnO exposure than after air exposure, indicating an initial enhanced release of cytokine from the cells of origin after inhalation of zinc oxide in spite of the absence of a symptoms or febrile response. The IL-6 responses comparing these two groups, were blunted and came somewhat earlier in the chronically exposed sheet metal workers. Compared with the pattern of IL-6 response in naive subjects, the chronically exposed workers' mean maximum level at 3 hours was slightly higher, but had begun to return toward baseline at 6 hours, rather than continuing to a higher level at 6 hours, as seen in naive subjects.

B. To determine whether the inflammatory pulmonary response, which is known to occur after a single exposure to zinc oxide fume, persists following repeated exposure.

This series of studies was also carried out with the identical protocol to the dose response study except that naive subjects were randomized to either one exposure or three daily exposures at 5.0 mg/m^3 for two hours, followed by a single broncho-alveolar lavage (washing of the lining fluid of the lung through a flexible fiberoptic bronchoscope) the day following the last exposure.

Naive subjects were chosen for this study based on the presence of a positive symptom and/or fever response to one zinc oxide exposure (since tolerance to exposure can not be demonstrated in individuals who do not respond at 5.0 mg/m^3). The development of tolerance to repeated exposures for two hours on three sequential days vs. one day were compared in two groups of naive subjects. For a group of five subjects who displayed a rise in oral temperature $\geq$ 1 degree F., the maximum change in temperature from baseline after three sequential challenges was progressively lower with each challenge (Figure 13). BAL cell counts were also compared in subjects who received 1 day exposure vs. subjects who received 3 days exposure. Analysis of those results and correlations with other indices of response to serial challenge have not been completed at the time of writing of this summary, but will be reported in future scientific papers.

C. To determine whether adaptation of clinical responses involves changes in metallothionein gene expression and the cytokines that may, at once, produce symptoms and regulate metallothionein synthesis, we will determine whether:

1. galvanized sheet metal workers and thermal cutters have higher inducible levels of peripheral blood lymphocyte metallothionein than naive controls.

Metallothionein is an acute phase reactant protein which is rapidly induced in a number of species in response to acute exposure to zinc oxide and other metals. Its physiologic roles are uncertain, but a role in the binding and excretion of zenobiotic metals, and a role as an anti-oxidant in the lung, have been postulated.

We did not demonstrate induction of metallothionein message (messenger RNA) in our naive subjects, although parallel animal studies in our laboratories using homogenized lung tissue lead us to believe that induction of this carrier protein may be occuring in lung tissue of subjects exposed under conditions such as ours.

In a preliminary study described in the application to this grant, a small group of acutely exposed individuals demonstrated an induction of metallothionein messenger RNA (mRNA) by in vitro cadmium chloride stimulation of peripheral blood lymphocytes from acutely exposed individuals. Subsequent to the development of the above hypothesis, studies of peripheral blood lymphocyte metallothionein induction in a chronically exposed industrial population, performed in Dr. Garte's laboratory, indicated that peripheral lymphocytes were not the appropriate tissue to examine for induction of metallothionein after acute inhalation exposure to zinc oxide, because they showed no response in adults chronically exposed to inducing metals in a large epidemiologic study in Eastern Europe.

This was confirmed in a study of four naive subjects whose peripheral blood lymphocyte mRNA was studied at 0, 3, 6, and (in 3 subjects) 24 hours after ZnO inhalation. No increase over baseline metallothionein mRNA was seen in this group when expressed as either absolute value (Figure 7A), or per cent change (Figure 7B).

We therefore developed an approach to assay the respiratory tract epithelium to more closely approximate the induction of metallothionein seen in respiratory tissue of several species seen in our other studies, (Cosma G, Fulton H, DeFeo, Gordon T: Metallothionein and heme oxygenase gene expression following ozone and zinc oxide exposure. *Toxicol Appl Pharmacol* 1992; 117:75-80). A new method for measuring metallothionein messenger RNA induction using a reverse transcriptase polymerase chain reaction was developed by Dr. Garte, and applied to pre-and post zinc oxide exposure nasal epithelial cells harvested by gentle cytology brushing of the nasal septum followed by immediate freezing of cells for later analysis.

Using this approach to assay a part of the upper respiratory system through which inhaled zinc oxide passes during our exposure protocol, we were not able to demonstrate induction of metallothionein

either by a dose response study.

Figure 8A shows that mean levels of epithelial metallothionein mRNA did not differ after 0, 2.5, and 5 mg ZnO. Figure 8B shows the same data expressed as the per cent change in nasal epithelial mRNA after the same 0, 2.5, and 5 mg/m^3 exposure.

In parallel studies Drs. Gordon, Chen, and Cosma measured induction of target genes in rodent models following single and repeated exposures to ZnO fume. Mice and rats were exposed for 3 hours to air or 1 to 5 mg/m^3 ZnO, lungs excised, total RNA isolated, and specific mRNA levels quantitated by standard slot blot analysis. In Sprague-Dawley rats, lung metallothionein mRNA values increased 8-fold over control levels immediately after exposure to 5 mg/m^3 ZnO. In Swiss mice lung metallothionein mRNA values increased 30 fold immediately following a single exposure to Zn and returned to control values by 48 hours. Thus, the significantly greater metallothionein induction in Swiss mice suggests that important inter-species differences must be considered in the interpretation of animal toxicity data regarding ZnO fume inhalation. Animal studies also strongly suggest the likelihood that metallothionein induction in man may be seen in lung tissue as well.

2. Sequential exposure of naive subjects to 5 mg/m^3 zinc oxide induces clinical tolerance and modulates metallothionein gene expression in peripheral blood and pulmonary macrophages.

a. Clinical tolerance.

As noted above in the dose response study of naive subjects, variability in response at the doses studied is such that some subjects have no significant response on exposure compared with control days, while others have marked symptoms and/or fever responses. In this portion of the study, we also used non-exposed naive subjects to study the phenomenon of tolerance long described in the anecdotal literature of metal fume fever, and subsequently demonstrated in an early human exposure study (Drinker P, Thomson R, Finn JL. Metal fume fever: IV. Threshold doses of zinc oxide, preventive measures, and the chronic effects of repeated exposure. J Ind Hyg 1927; 10:331-345).

We selected for the study of tolerance to repeated challenge subjects who demonstrated a response to initial challenge at the maximal dose of 5.0 mg/m^3 zinc oxide.

These "responders" demonstrated a progressive diminution in both the oral fever response and the total symptom score after the second and third daily exposure, illustrating the rapidity with which tolerance to zinc oxide is induced (Figure 13).

Because previous studies have demonstrated the presence of an asymptomatic inflammatory response in the lungs within 24 hours after zinc oxide inhalation, we sought to determine whether adaptation occurs in the pulmonary inflammatory response, or at some stage "downstream" to the initial cellular response seen in lung lining fluid.

Bronchoalveolar lavage (BAL) was performed after one 5.0 mg/m^3 exposure in one group of

naive subjects (n=19) and after three sequential daily 5.0 mg/m^3 exposures in the second group of naive subjects (n=10). In subjects who underwent three exposures, there was a reduction in the blood IL-6 response as well as in the symptom and temperature response, indicating that IL-6 may be important to the mechanistic sequence of events between lung inhalation of zinc oxide and the development of fever and symptoms. Results of BAL cell counts and cytokines from these serially challenged subjects were also studied but have not been analyzed at the writing of this summary, and will be reported in future scientific publications of this research.

To explore the possible development of tolerance in parallel studies in our lab, Drs. Gordon, Chen, and Cosma, exposed Swiss mice to 5 mg/M^3 for 3 hours per day for 5 days, allowed 5 days rest, and then re-exposed to air or ZnO for 3 hours. Mice re-exposed to ZnO exhibited a significantly greater inducibility of lung metallothionein gene expression than naive mice receiving a single ZnO exposure. Because of the proposed physiologic role for metallothionein in zinc homeostasis, these studies suggested a molecular basis for the development of tolerance reported in welders, sheet metal workers, and others repeatedly exposed to zinc oxide. In collaboration with Dr. Stuart Horowitz (Sterling Winthrop Hospital) the next series of studies examined the response of transgenic mice (with either 56 or 112 extra copies of the metallothionein gene) or their litter mates to ZnO inhalation. As determined by examination of biochemical and cellular parameters in lavage fluid, the increased number of copies of the metallothionein gene did not protect the mice against a single exposure to 1 mg/m^3 ZnO. To examine whether these extra copies of the metallothionein gene need to be turned on in order to provide protection, transgenic mice (56 extra copies) were exposed to 1 mg/m^3 ZnO for 5 days and then re-exposed after a 5 day rest. These animals exhibited a significant decrease in the response to ZnO inhalation as opposed to control animals exposed in an identical fashion. Thus, the expression of additional metallothionein protein, and not just the presence of extra gene copies, appeared to be necessary for the induction of tolerance.

3. The pattern of cytokine release in the blood of the chronically exposed workers and the blood and the bronchoalveolar lavage of naive subjects corresponds to symptomatic responses and the level of metallothionein gene expression.

As noted above, both naive subjects and chronically exposed sheet metal workers showed an increase in peripheral blood levels of IL-6 (but not TNF) in our studies. When naive subjects were challenged on three separate days at the OSHA PEL of 0.5 mg/m*3*, their blood IL-6 levels after the third challenge were lower than in subjects after a single challenge. Analysis of correlation of symptom and fever responses to blood and BAL levels of cytokines has not been completed at the time of writing this summary, but will be reported in future scientific publications of this research.

V. ACKNOWLEDGEMENT

This research could not have been accomplished without the support, close cooperation and volunteer assistance of the Sheet Metal Workers International Association. Particular thanks are due to Mr. James Neary, business manager of Local #40, and Mr. Guy DiMaggio. Many members of this organization generously supported this occupational health research with their time and expertise.

VI. LIST OF PRESENT AND POSSIBLE FUTURE PUBLICATIONS

a. Abstracts

Zinc oxide at current permissible exposure level induces symptoms, fever, and rise in plasma IL-6. JM Fine, T Gordon, LC Chen, P Kinney, G Falcone, W Beckett. Am J Respir Crit Care Med 1995; 151(4) A259, April 1995.

Serial challenge with ultrafine zinc oxide induces rapid adaptation to metal fume fever in normal subjects. W Beckett, T Gordon, LC Chen, P Kinney, G Falcone, S Garte, and J Fine. Sixth International Meeting on the Toxicology of Natural and Man-Made Fibrous and Non-Fibrous Particles, Lake Placid, NY, September 15-18, 1996.

b. Papers - published or submitted

Metal fume fever. Gordon T, Fine JM. Occupational Medicine: State of the Art Reviews 1993; 8:505-517.

Metal Fume Fever: Characterization of clinical and plasma IL-6 responses in controlled human exposures to zinc oxide fume. JM Fine, LC Chen, P Kinney, G Falcone, and WS Beckett. Submitted, Occupational and Environmental Medicine, 1996.

c. Papers - planned

Tolerance to acute challenge with ultrafine zinc oxide fume in sheet metal workers with asymptomatic chronic exposure to low-level zinc oxide.

Induction of tolerance to fever, symptoms, and inflammatory responses of zinc oxide with 2-hour serial daily exposure to zinc oxide fume at the OSHA PEL.

VII. Figure Legends

Figure 1. Zinc oxide concentrations measured at 20 minute intervals throughout the 2 hour exposures. The target exposure concentrations were 2.5 mg/m^3 and 5.0 mg/m^3, the current OSHA PEL.

Figure 2. Mean (±SE) changes in oral temperature from the pre-exposure baseline following inhalational exposures to air, 2.5 mg/m^3, and 5 mg/m^3 zinc oxide. * denotes significant difference ($p < 0.05$) from baseline.

Figure 3. Mean (±SE) changes in total symptom score from pre-exposure baseline following inhalational exposures to air, 2.5 mg/m^3 and 5 mg/m^3 zinc oxide. total symptom score represents the sum of 13 different symptoms scores. *denotes significant difference, ($p < 0.05$) from baseline.

Figures 4A-C. Mean ±SE changes in cough (A), fatigue (B), myalgia (C) scores from pre-exposure baseline following inhalational exposures to air, 2.5 mg/m^3, and 5 mg/m^3 zinc oxide. *denotes significant difference ($p < 0.05$) from baseline.

Figure 5. Mean (± SE) changes in IL-6 (pg/ml) from pre-exposure baseline following inhalational exposures to air, 2.5 mg/m^3 and 5 mg/m^3 zinc oxide. *denotes significant difference ($p < 0.05$) from baseline.

Figure 6. Mean (±SD) peripheral blood white cell count before and 24 hours after exposure to 5.0 mg/m^3 zinc oxide. * denotes statistically greater levels after exposure.

Figures 7A-B. Peripheral blood lymphocyte metallothionein messenger RNA immediately after and 3, 6, and 24 hours after exposure to zinc oxide, expressed as mean (A) and mean per cent change (B).

Figures 8A-B. Nasal epithelial cell metallothionein messenger RNA after exposure to 0, 2.5, and 5.0 mg/m^3 zinc oxide, expressed as mean (A) and per cent change (B).

Figure 9. Schematic of sheet metal fabrication shop where air levels of zinc oxide were measured.

Figure 10. Air levels of zinc oxide in two sheet metal fabrication shops (shop A diagrammed in fig.9). Numbers adjacent to areas in Shop A correspond with numbered locations in Fig. 9.

Figure 11. 24 hour urine collection volume and zinc concentrations for sheet metal fabrication shop workers prior to laboratory zinc oxide exposure as chronically exposed workers. These values represent recent (24-48 hour) zinc exposure by inhalation and dietary intake.

Figure 12. Comparison of symptoms in chronically exposed sheet metal workers after 0 mg/m^3 (control) exposure and 5.0 mg/m^3 exposure to zinc oxide.

(Figure legends continued next page)

(Figure Legends, cont'd)

Figure 13. Maximum change in oral temperature from pre-exposure baseline in five previously unexposed normal subjects who responded with a rise in oral temperature to an initial challenge with 5.0 mg/m^3 zinc oxide, and underwent two further exposures at the same concentration on subsequent days.

Figure 14. Change in total symptom score from pre-exposure baseline in nine normal subjects with three daily exposures to zinc oxide at 5 mg/m^3, two hours exposure. Mean cumulative symptom score fell with two subsequent exposures, and was significantly lower after exposure day 3 than after exposure day 1.

FIGURE 1

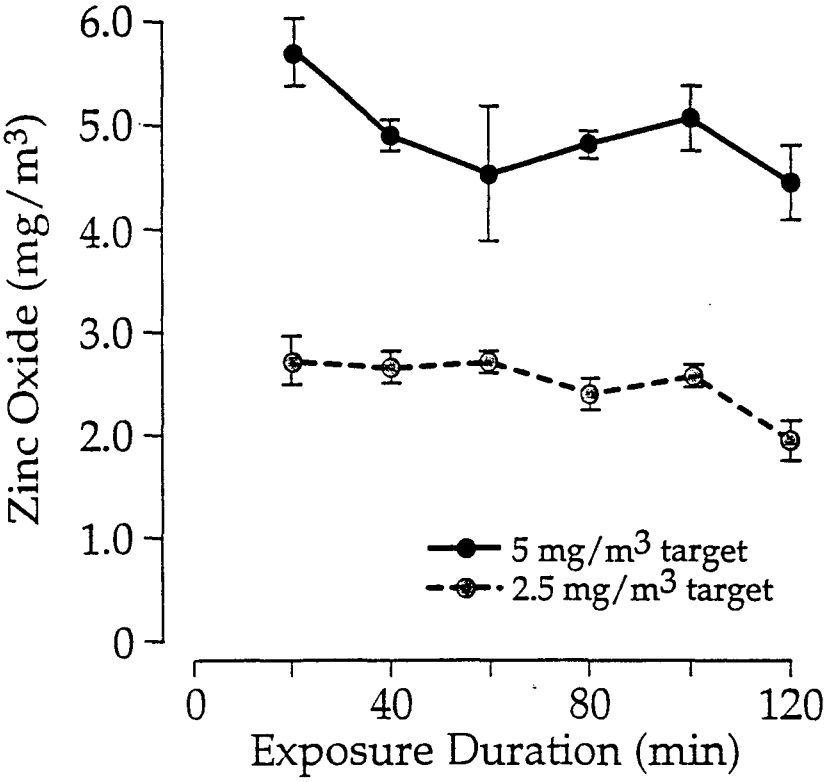

6.0
5.0
4.0
3.0
2.0
1.0
0
Zinc Oxide (mg/m3)
5 mg/m3 target
2.5 mg/m3 target
0
40
80
120
Exposure Duration (min)

FIGURE 2

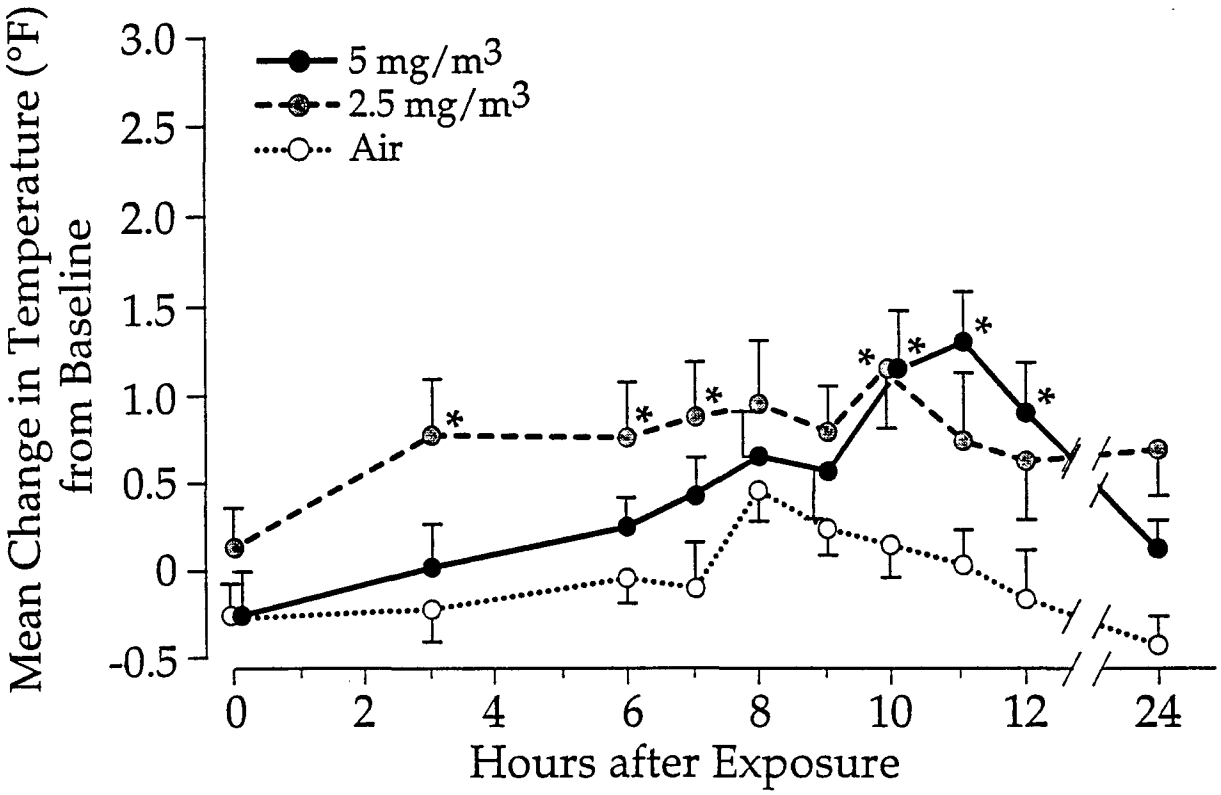

Mean Change in Temperature (°F) from Baseline
3.0
2.5
2.0
1.5
1.0
0.5
0
-0.5
5 mg/m3
2.5 mg/m3
Air
*
0
2
4
6
8
10
12
24
Hours after Exposure

FIGURE 3

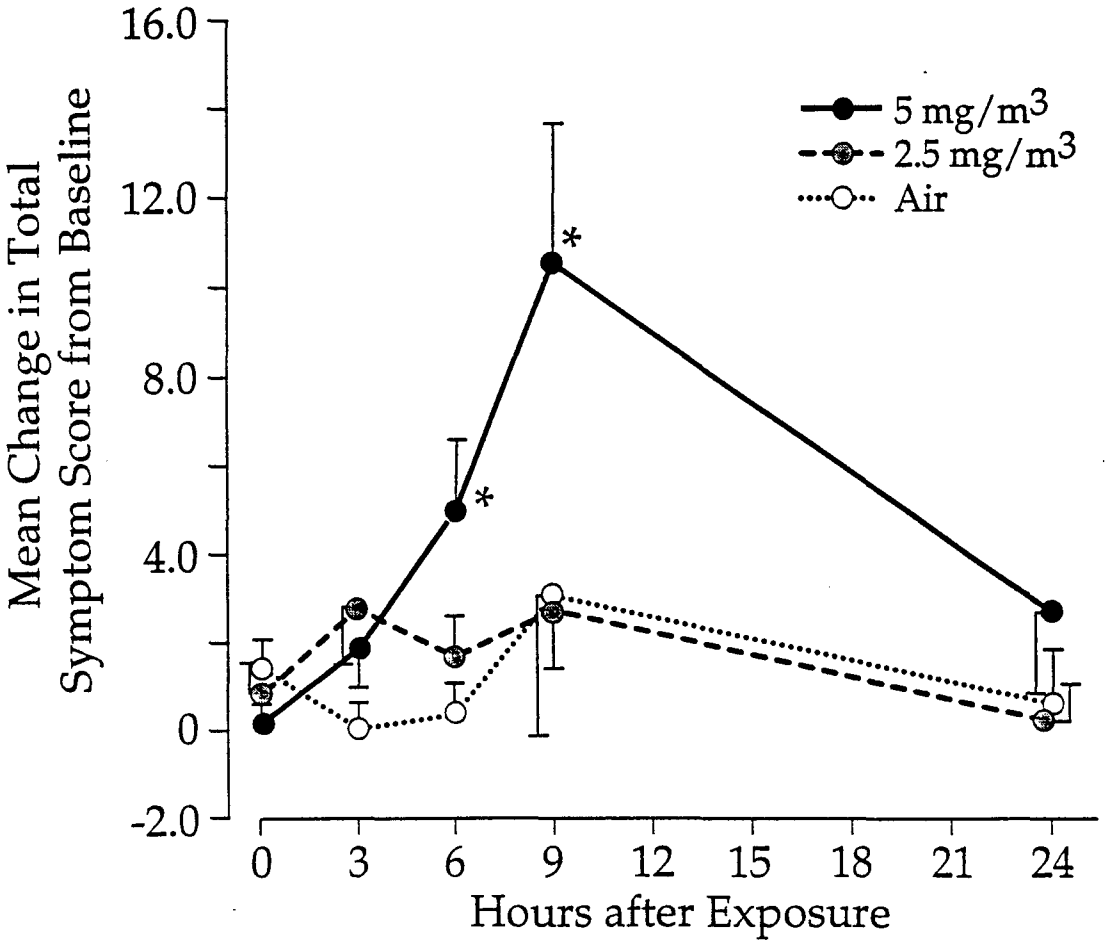

16.0
12.0
8.0
4.0
0
-2.0
Mean Change in Total Symptom Score from Baseline
5 mg/m3
2.5 mg/m3
Air
*
*
0
3
6
9
12
15
18
21
24
Hours after Exposure

FIGURE 4a

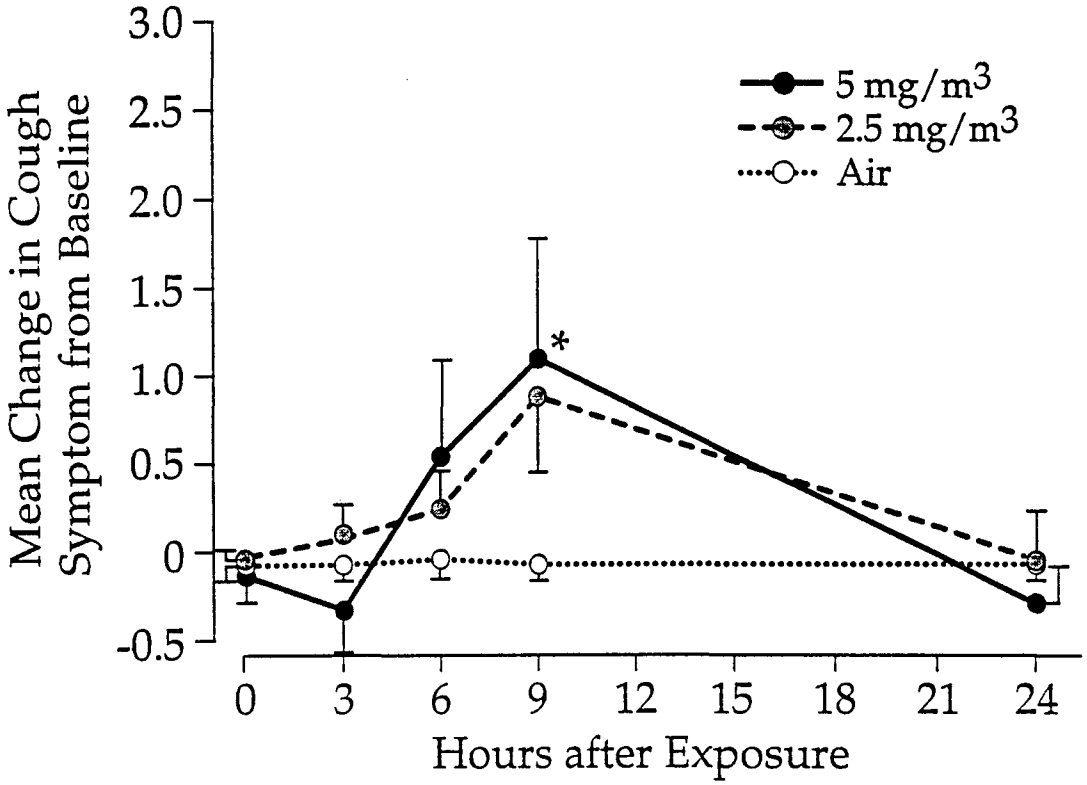

Mean Change in Cough Symptom from Baseline
3.0
2.5
2.0
1.5
1.0
0.5
0
-0.5
0
3
6
9
12
15
18
21
24
Hours after Exposure
5 mg/m3
2.5 mg/m3
Air
*

Figure 4b

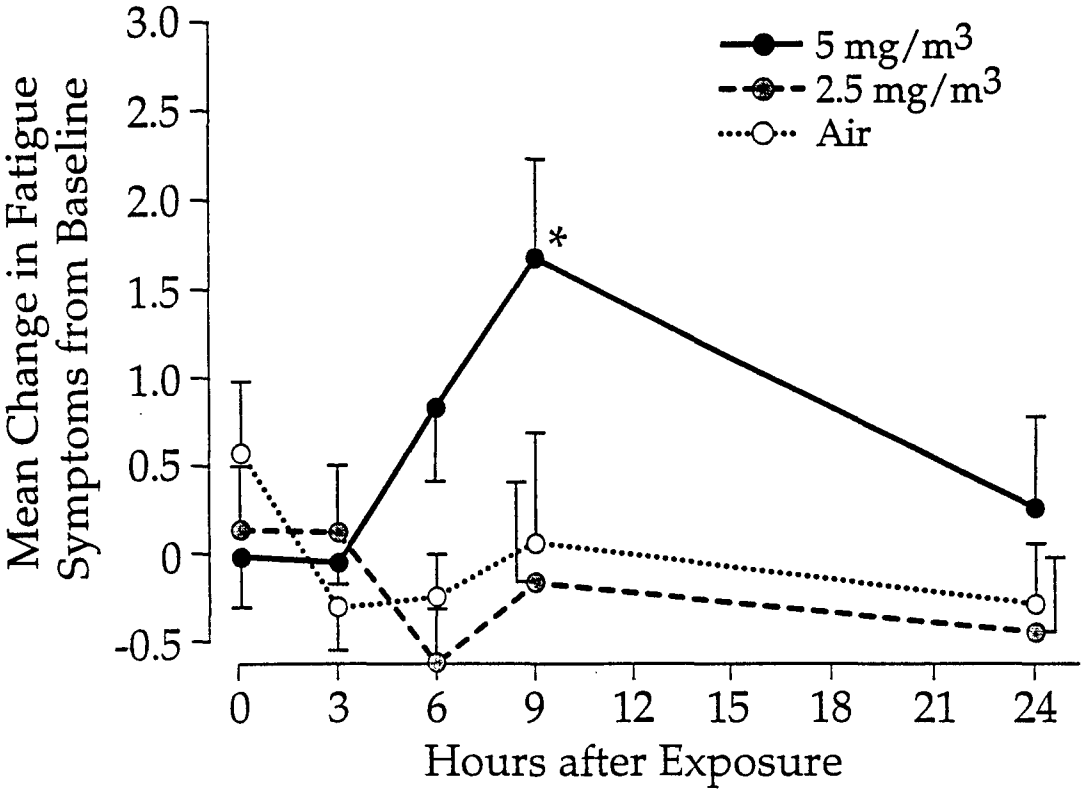

Mean Change in Fatigue Symptoms from Baseline
3.0
2.5
2.0
1.5
1.0
0.5
0
-0.5
0
3
6
9
12
15
18
21
24
Hours after Exposure
5 mg/m3
2.5 mg/m3
Air
*

Figure 4c

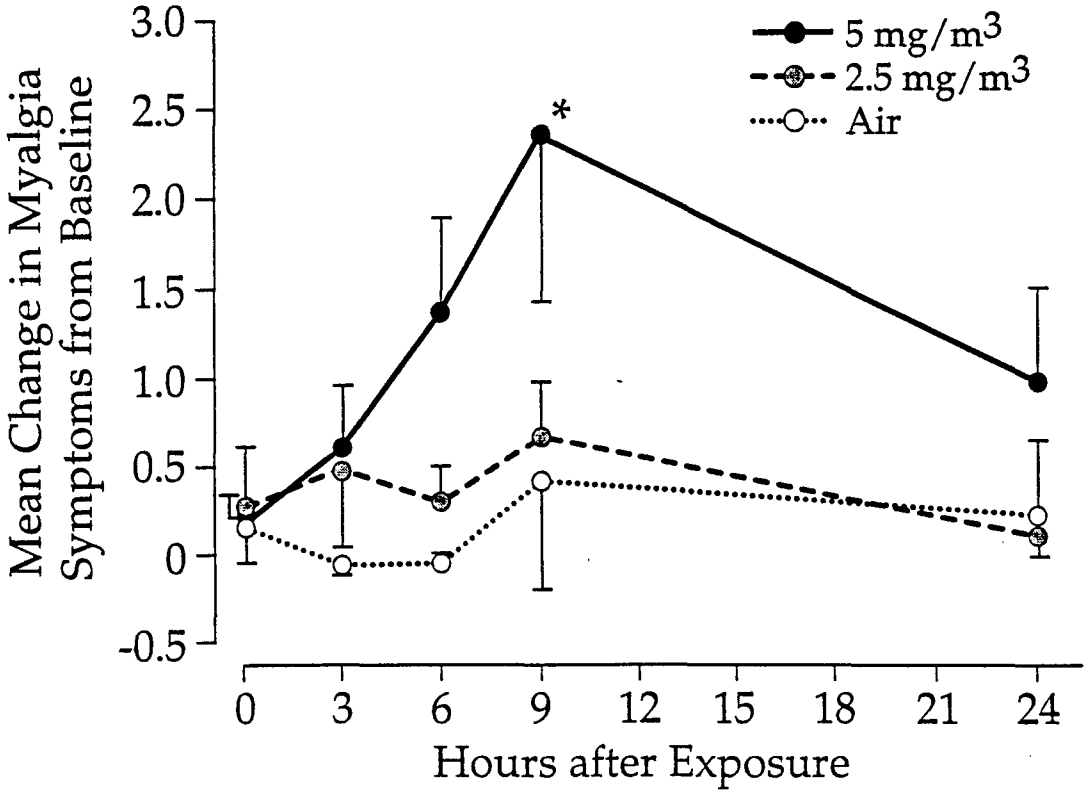

Mean Change in Myalgia Symptoms from Baseline
3.0
2.5
2.0
1.5
1.0
0.5
0
-0.5
*
5 mg/m3
2.5 mg/m3
Air
0
3
6
9
12
15
18
21
24
Hours after Exposure

Figure 5

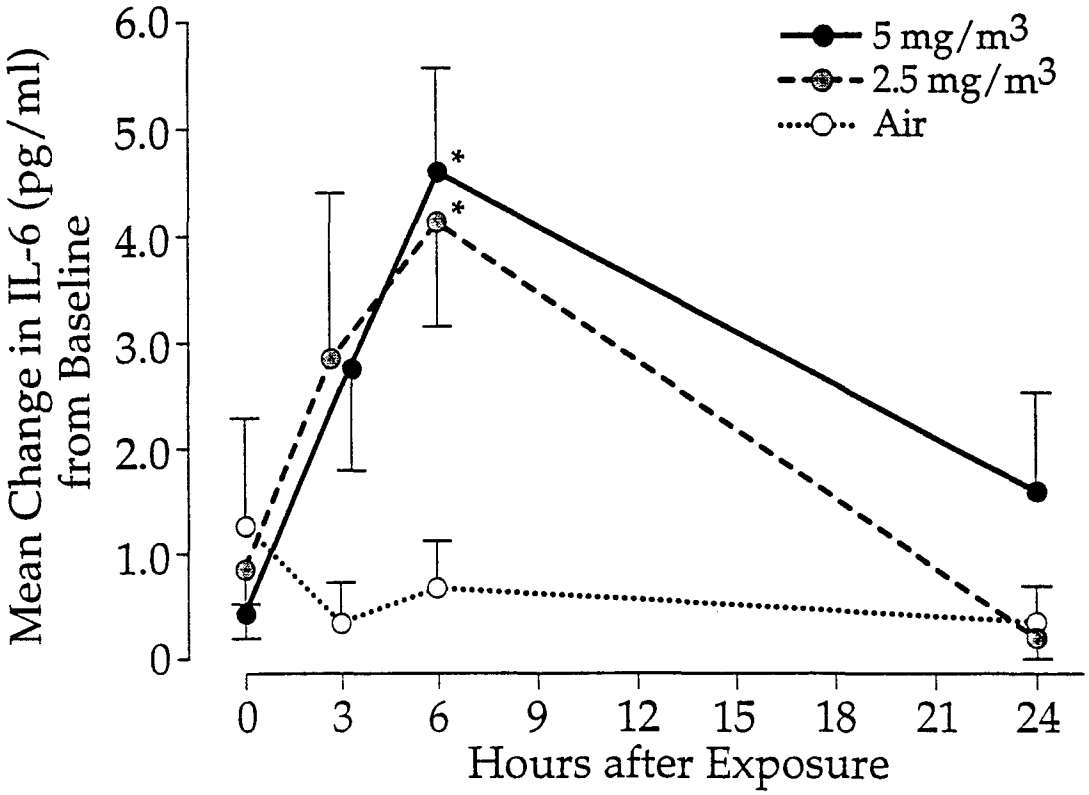
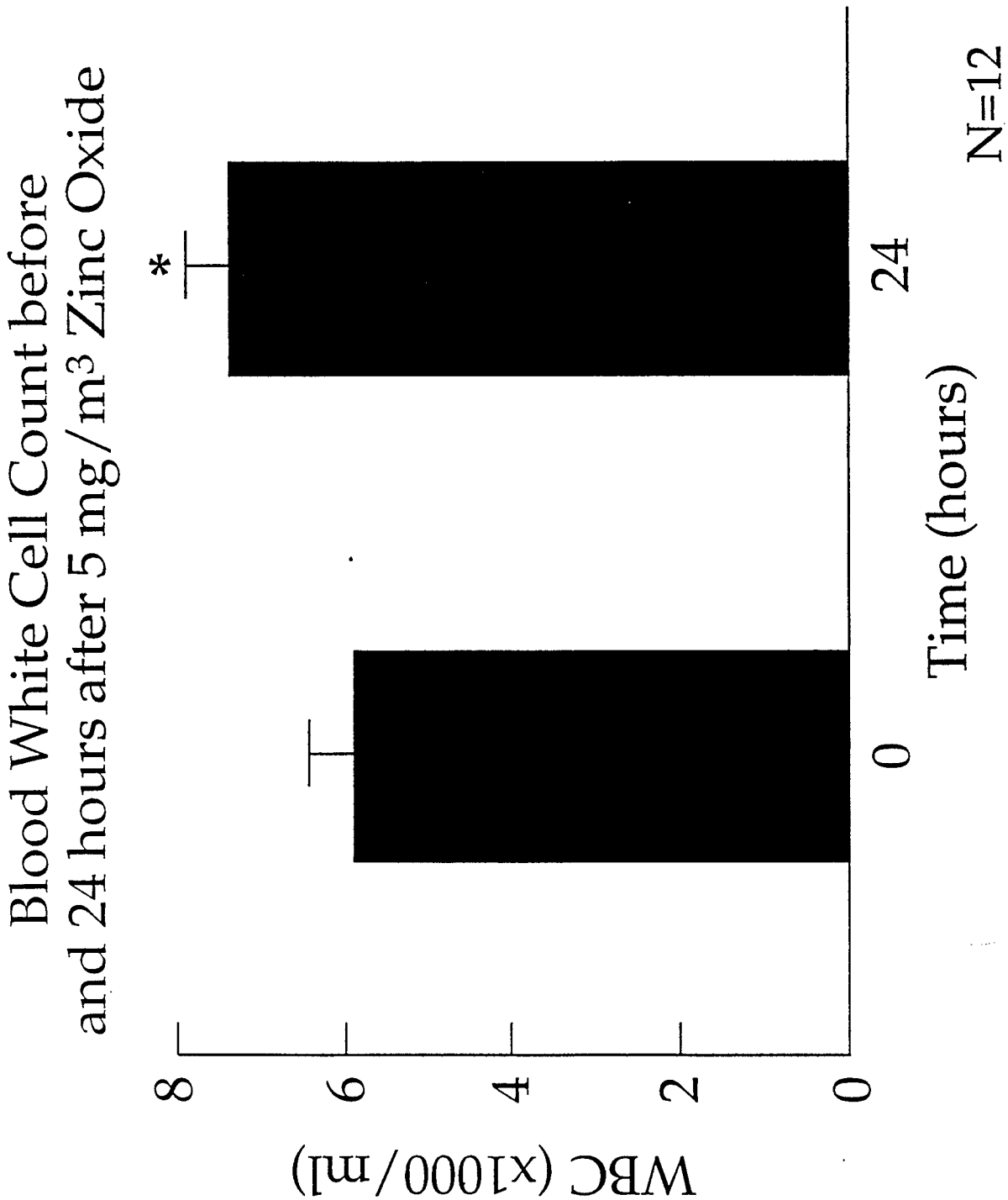
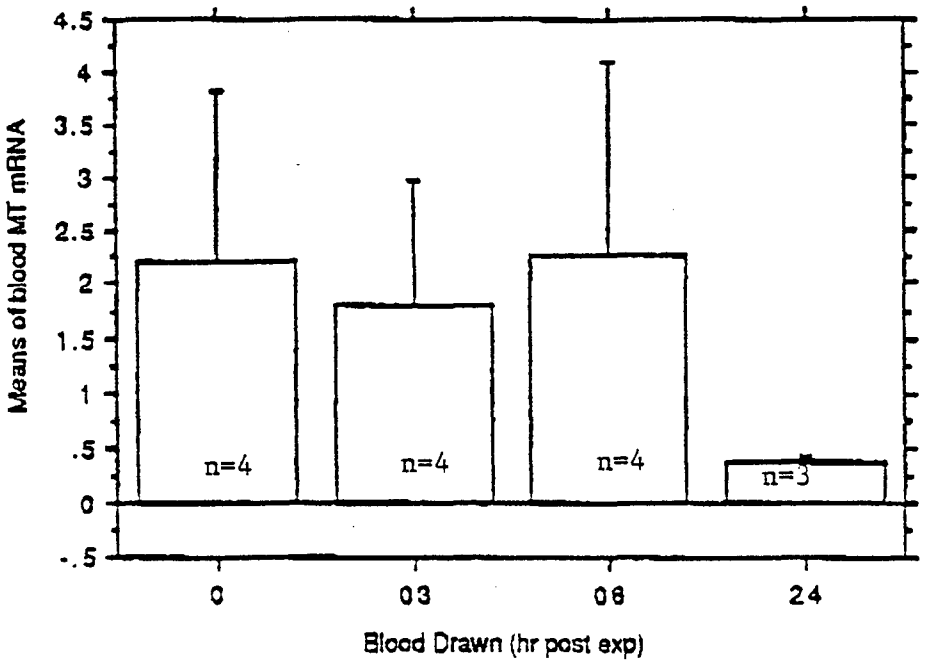
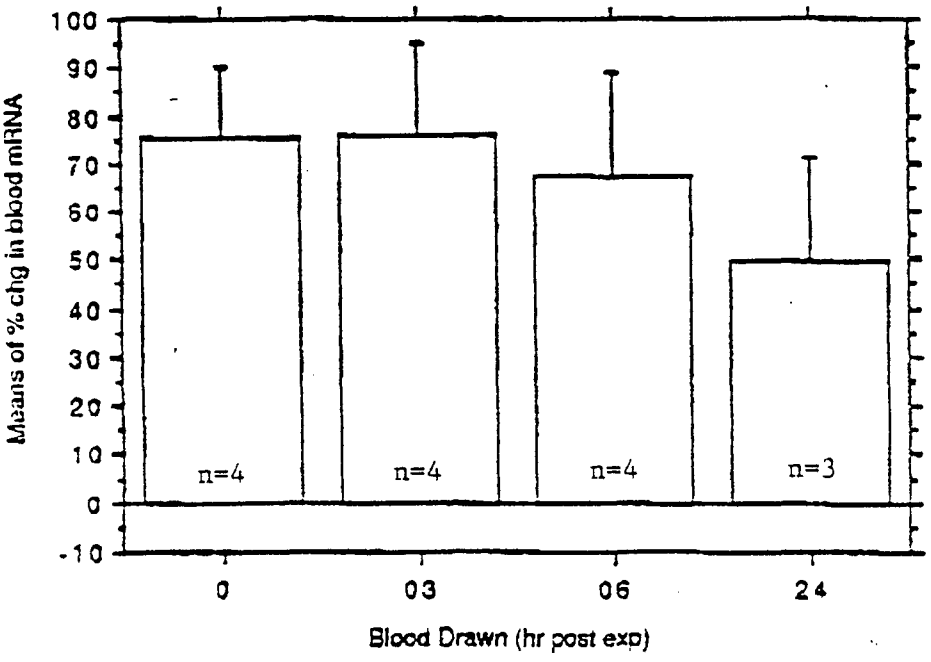

Mean Change in IL-6 (pg/ml) from Baseline
6.0
5.0
4.0
3.0
2.0
1.0
0
5 mg/m3
2.5 mg/m3
Air
*
*
0
3
6
9
12
15
18
21
24
Hours after Exposure

Figure 6

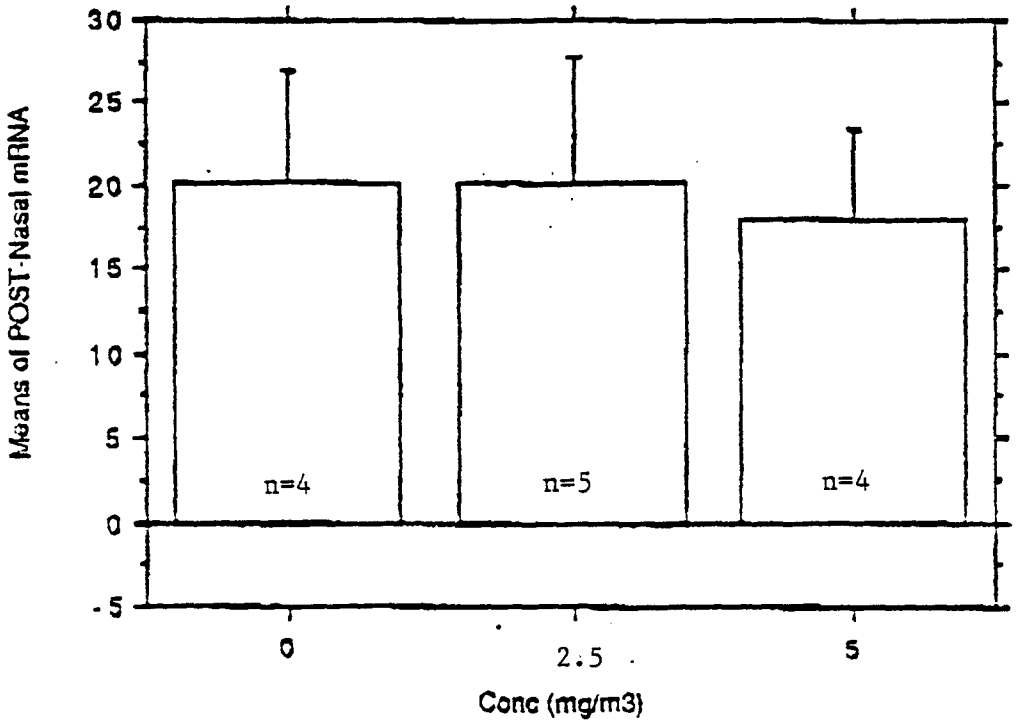

Blood White Cell Count before
and 24 hours after 5 mg/m3 Zinc Oxide
WBC (x1000/ml)
0
2
4
6
8
*
0
24
Time (hours)
N=12

FIGURES 7A-B


Means of blood MT mRNA
4.5
4
3.5
3
2.5
2
1.5
1
.5
0
-.5
n=4
n=4
n=4
n=3
0
03
06
24
Blood Drawn (hr post exp)


Means of % chg in blood mRNA
100
90
80
70
60
50
40
30
20
10
0
-10
n=4
n=4
n=4
n=3
0
03
06
24
Blood Drawn (hr post exp)

FIGURES 8A-B


Means of POST-Nasal mRNA
30
25
20
15
10
5
0
-5
n=4
n=5
n=4
0
2.5
5
Conc (mg/m3)

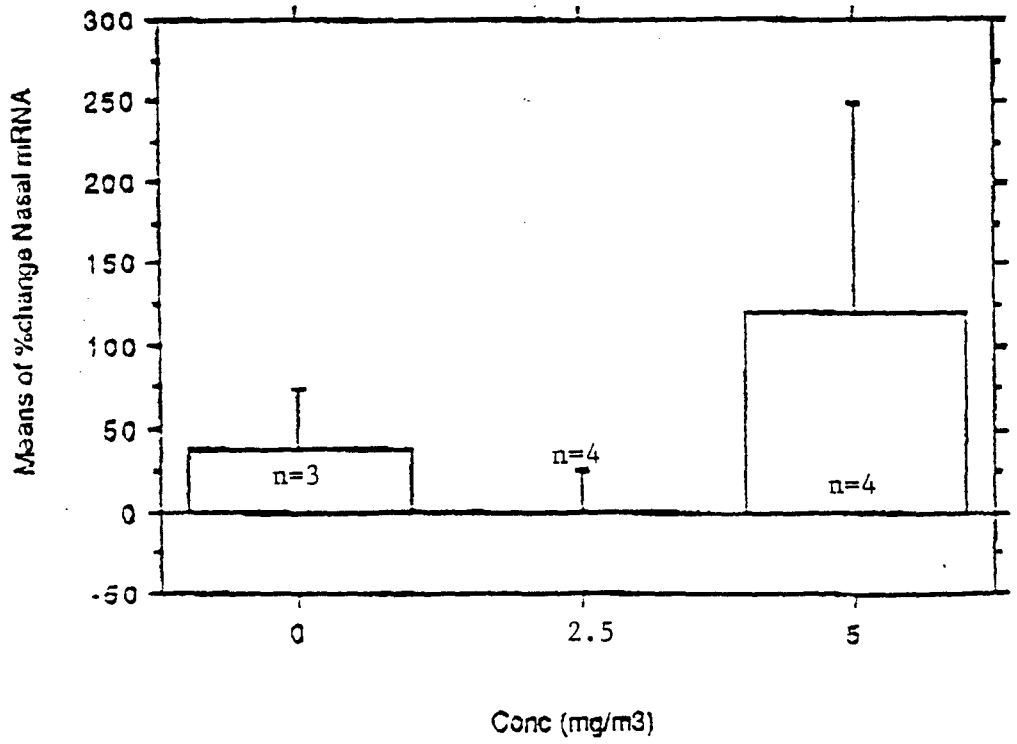
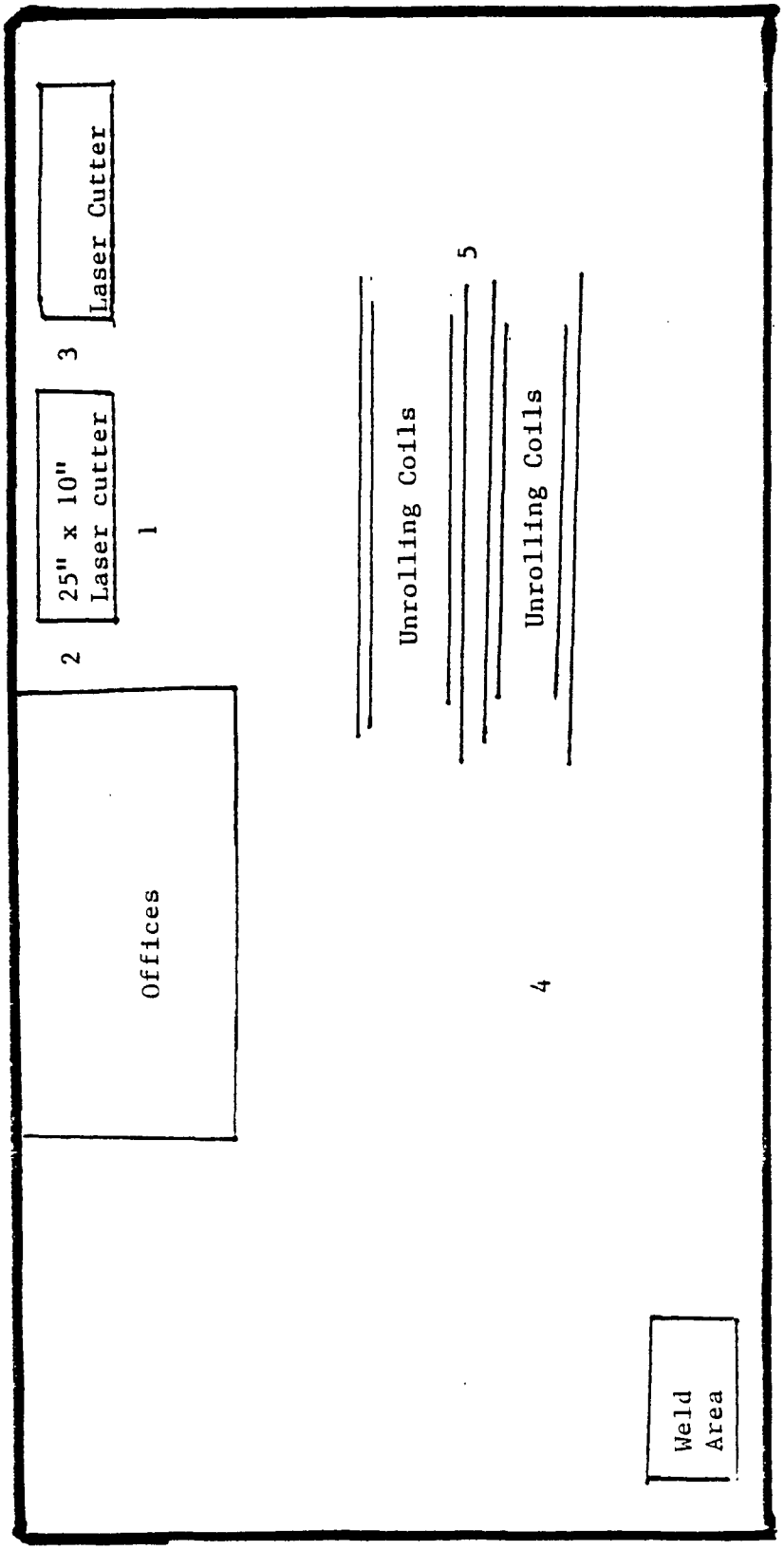

Means of %change Nasal mRNA
300
250
200
150
100
50
0
-50
n=3
n=4
n=4
0
2.5
5
Conc (mg/m3)

Figure 9


Offices
2
25" x 10"
Laser cutter
1
3
Laser Cutter
Unrolling Coils
5
Unrolling Coils
4
Weld
Area
Shop A
125' x 50' x 10'

ZINC OXIDE AIR SAMPLES FROM TWO SHEET METAL SHOPS (OSHA PEL 5.0 MG/M^3)

Sample Location	Zinc Oxide Concentration mg/m^3
SHOP A	0.12
1. Personal sample, cutter	
2. Area sample, 5 ft from end of cutter	0.09
3. Area sample, between cutters	0.10
4. Area sample 30 ft from cutters	0.04
5. Area sample, roller machine console, 20 feet away	0.04
SHOP B	
Personal sample, plasma cutter operator	0.04
Area sample, 3-4 ft from cutter	<0.03
Area sample, 3 ft from side	<0.03
Area sample, 8 ft from cutter	<0.03
Area sample, 20 ft away	0.03

24 Hour Urinary Zinc Excretion* In Sheet Metal Workers Occupationally Exposed to Zinc Oxide

Subject	Urine Volume (ml)	Urine Zinc (mcg)
1	2490	1394
2	2260	407
3	1175	975
4	1190	1035
5	1800	1278
6	1100	1243
7	700	770
8	1725	1259
9	1650	429

*Laboratory reference range 150-1250 mcg/24 hours

FIGURE 11

FIGURE 12

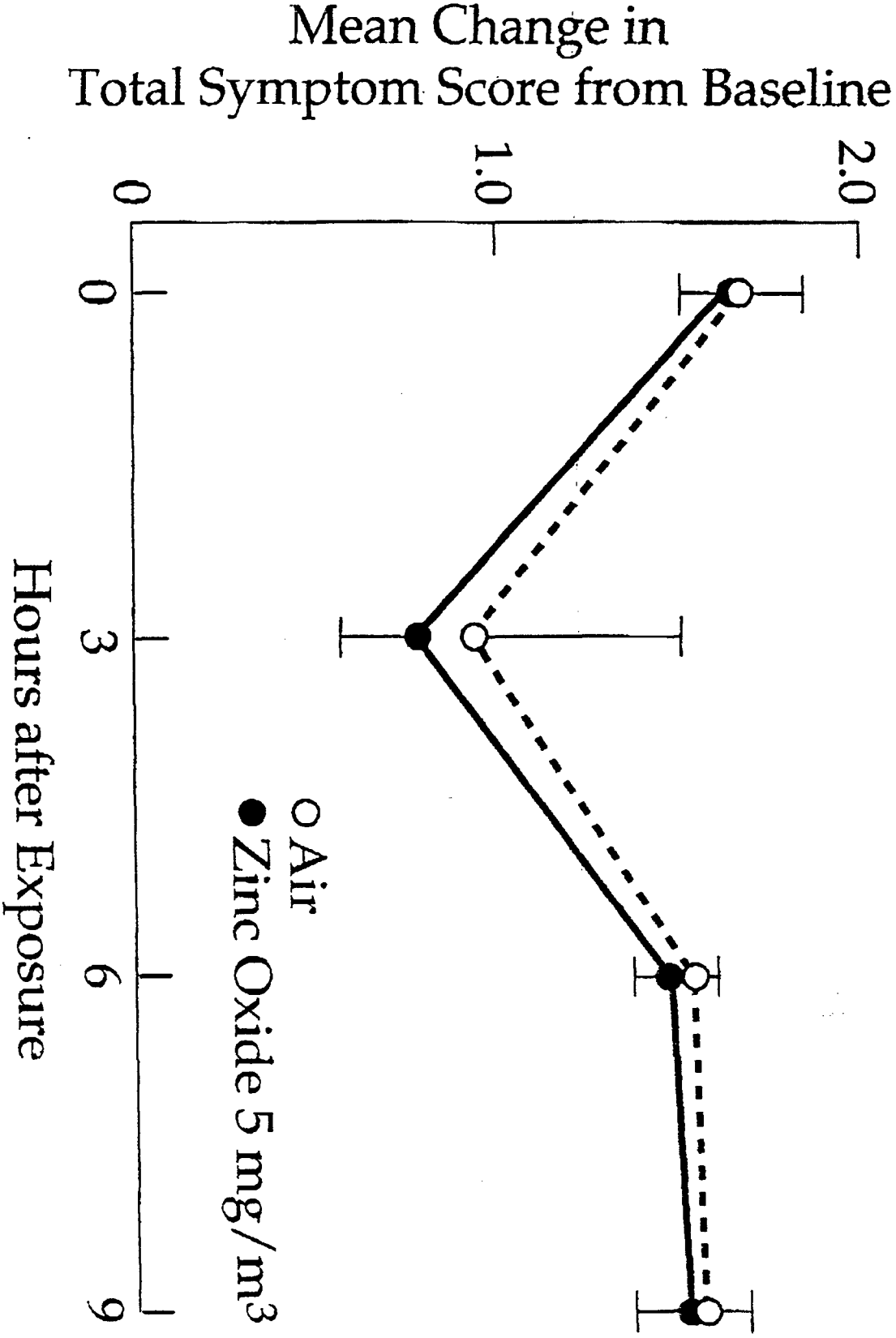

Mean Change in
Total Symptom Score from Baseline
0
1.0
2.0
0
3
6
9
Hours after Exposure
○ Air
● Zinc Oxide 5 mg/m3

FIGURE 13

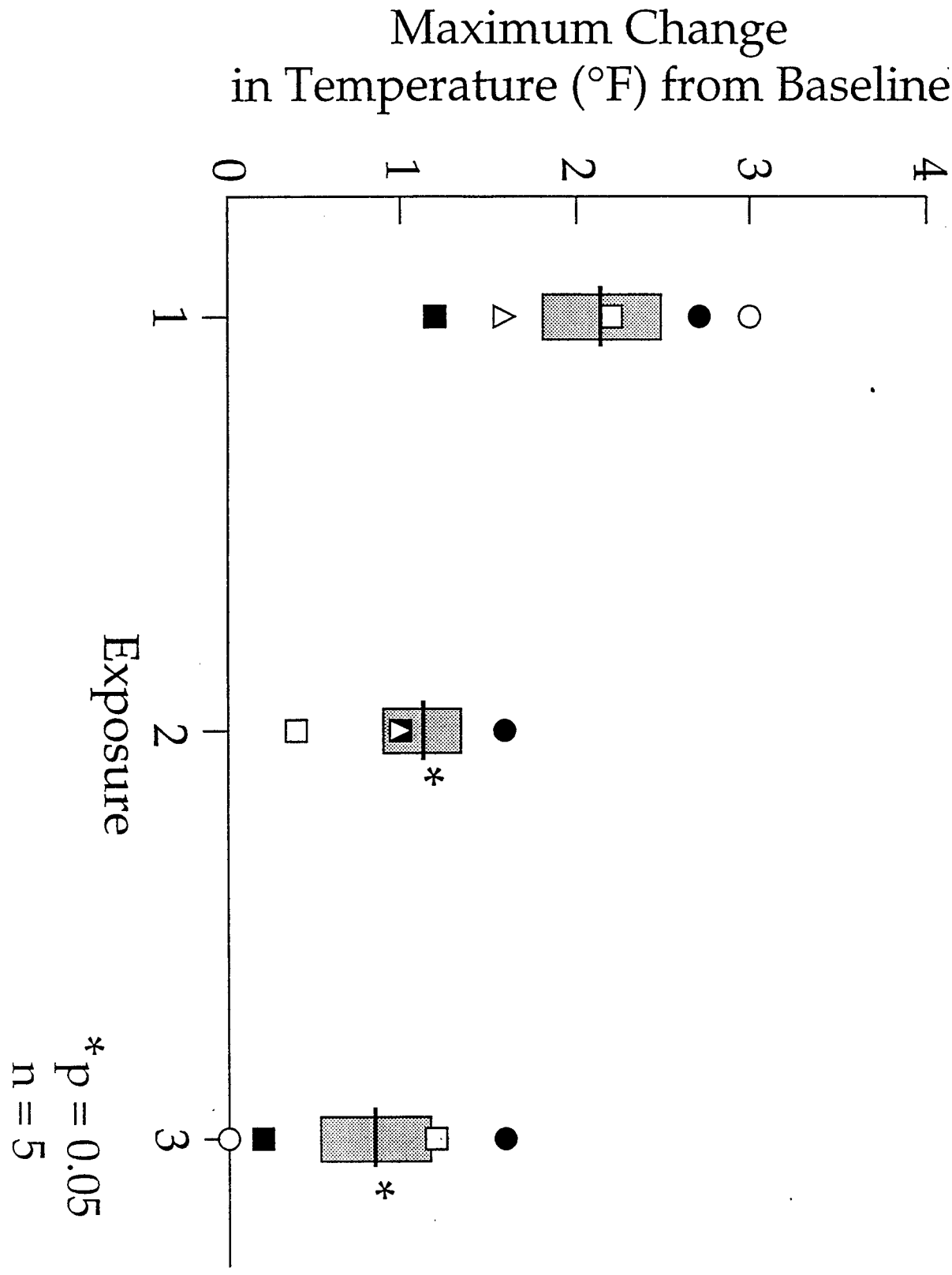
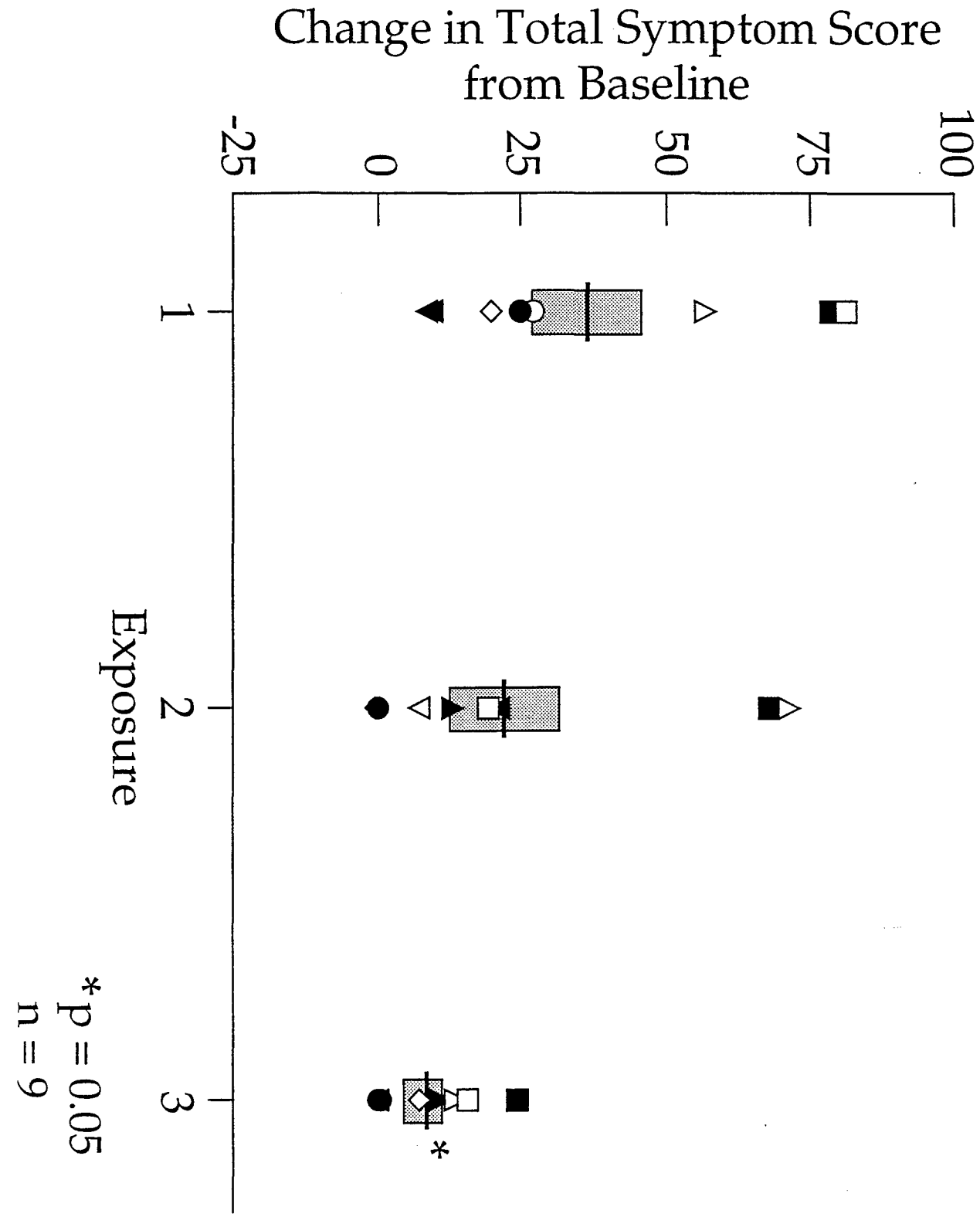

Maximum Change
in Temperature (°F) from Baseline
0
1
2
3
4
Exposure
1
2
3
*
*
*p = 0.05
n = 5

FIGURE 14


Change in Total Symptom Score from Baseline
-25
0
25
50
75
100
Exposure
1
2
3
*
*p = 0.05
n = 9

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Lake Placid, New York, USA, September 15 - 18, 1996

SERIAL CHALLENGE WITH ULTRAFINE ZINC OXIDE INDUCES RAPID ADAPTATION TO METAL FUME FEVER IN NORMAL SUBJECTS. W Beckett*, T Gordon, L C Chen, P Kinney, G Falcone, S Garte, and J Fine. Department of Medicine, Yale University School of Medicine, New Haven, CT; Norwalk Hospital, Norwalk CT, and New York University Institute of Environmental Medicine, Sterling Forest, NY, USA.

Metal fume fever is an acute febrile systemic illness from inhaled, freshly generated zinc oxide fume. A "reverse sensitization" or tolerance to repeated exposures has been prominent in the clinical description of this illness. Individuals who once experience the clinical illness become strikingly tolerant to subsequent similar exposures on return to work. The tolerance lasts as long as regular exposures continue, but can be lost after an interval away from work. To simulate workplace exposures to zinc oxide fume and the accompanying clinical entity of metal fume fever, nine never-exposed human subjects breathed fresh zinc oxide fume, generated from pure zinc in an electric furnace, for 2 hours at 5.0 mg/m^3 air concentration (the U.S. Occupational Safety and Health Administration Permissible Exposure Limit for 8 hours), 0.07 μm mass median aerodynamic diameter. Those who demonstrated a systemic response at this dose (fever and symptoms) went on to have two additional identical exposures at the same time of day on subsequent days. There was a progressive and significant decline over three serial exposures in the cumulative systemic symptom score, and the maximal elevation in oral temperature after exposure, indicating rapid induction of tolerance to the systemic response to inhaled ultrafine zinc oxide fume. We hypothesize that rapid induction of the endogenous metal binding protein metallothionein in the lungs of these subjects, as previously demonstrated in animal models, is partially protective against fever and systemic symptoms. (Supported by the US National Institute for Occupational Safety and Health OH02987).

1995 ABSTRACT FORM

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ZINC OXIDE AT CURRENT PERMISSABLE EXPOSURE LEVEL INDUCES SYMPTOMS, FEVER, AND RISE IN PLASMA IL-6.

J. M. Fine, T. Gordon*, L. C. Chen*, P. Kinney*, G. Falcone, W. Beckett¥. Norwalk Hospital, Norwalk, CT; *Institute of Environmental Medicine, NYU Medical Center, Tuxedo, NY; ¥Yale University School of Medicine, New Haven, CT.

Metal fume fever is an acute, self-limited illness characterized chiefly by fever and systemic symptoms caused by inhalation of freshly formed fume of metal oxides, most commonly zinc oxide (ZnO). Results from animal and preliminary human exposure studies have called into question whether the 5 mg/ m^3 8 hr time-weighted average permissable exposure level (PEL) set by OSHA for ZnO fume is sufficient to protect workers against metal fume fever. Also, while the symptomolgy is suggestive of a systemic cytokine mediated process, elevated cytokine levels have not been found in plasma of persons acutely exposed to ZnO. In order to test the safety of exposure to 5 mg/m^3 and to ascertain whether exposure to ZnO elevates cytokine levels, we exposed 12 resting naive subjects to air, 2.5 and 5 mg/m^3 of ZnO fume for 2 hrs. The mean (±SE) maximum rise in oral temperature at 6 to12 hrs after exposure was 1.35 ±0.3 °C after 5 mg/m^3 compared to 0.59 ±0.5 °C after air exposure ($p < 0.05$). The temperature was also elevated after exposure to 2.5 mg/m^3 ZnO (1.23 ± 0.32 °C), but was not significantly different from air values. In a parallel fashion, plasma interleukin-6 (IL-6) levels were significantly elevated following exposure to 5 mg/m^3 ZnO. IL-6 values (pg/ml) at pre-exposure and at 3 and 6 hrs post-exposure were 1.68 (±0.6), 2.81 (±0.7), and 2.76 (±0.7), respectively, on the air days, 1.16 (±0.49), 4.32 (±1.73) and 5.71 (±1.36) on the 2.5 mg/m^3 ZnO days, and 1.38 (±0.5), 4.41 (±1.2), and 6.42 (±1.1) on the 5 mg/m^3 ZnO days. ZnO exposure did not significantly affect TNF levels. Nine hrs after exposure to 5 mg/m^3 ZnO, subjects reported more myalgias, cough, and fatigue than after air ($p < 0.05$). Thus, inhalation of ZnO for 2 hrs at the current PEL of 5 mg/m^3 produces fever and symptoms along with elevation in plasma IL-6 levels.

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