1053 PULMONARY TOXICITY OF INDIUM PHOSPHIDE PARTICULATE IN B6C3F1 MICE AFTER OROPHARYNGEAL ASPIRATION

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This study was conducted to investigate the mechanism of indium phosphide (InP) induced pulmonary carcinogenesis and pleural hyperplasia in B6C3F1 mice. InP is a particulate or brittle metal used in the microelectronics industry. Chronic inhalation exposure of mice to InP particles caused alveolar/bronchiolar adenomas and carcinomas, and hyperplasia of the pleural mesothelium (NTP, 2000). Although fibers are known to cause lesions in the pleural mesothelium, pleural effects are uncommon after inhalation of a non-fibrous particulate. B6C3F1 mice (n=5/group) were dosed with water, 1mg/kg or 2 mg/kg InP by oropharyngeal aspiration (OA). Bronchoalveloar lavage (BAL) and pleural lavage (PL) fluids were collected 1, 3, 14 and 28 days post-aspiration. PL from the InP animals were similar to controls until day 28, where a dramatic increase in LDH, cell# and total protein was observed. BAL cell#, total protein and LDH were increased in a dose- and time-related manner. Lung histology was performed on day 28 and chronic active inflammation was observed. No pathology was observed in the mesothelium. BAL from the 1mg/kg InP group and control days 3 & 28 were analyzed by quantitative luminex analysis. Complex changes in cytokine levels were observed, including IL-2, IL-3, IL-4, IL-6 IL-10, IL-11, IL-12, IL-12p70, IL-17, IL-18, MCP(s), MIP-1(s) and others. Although no pathological changes in mesothelial cell architecture were observed on day 28, the environment of the pleural cavity was strikingly different. Thus more time may be needed to see the mesothelial hyperplasia that was observed in the 2-year study. This change in the pleural environment may be due to progressive pleural tissue damage by InP or secondary to lung injury. Future directions include cytokine analysis on PL samples after day 28, InP inhalation experiments and the investigation of InP mediated free radical generation.

1054 BUTADIENE SOOT INDUCES AUTOFLUORESCENCE AND ULTRASTRUCTURAL DAMAGE IN CULTURED HUMAN RESPIRATORY EPITHELIAL CELLS AND CAUSES INFLAMMATION IN MURINE AIRWAYS

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A terrorist act that has been repeatedly hypothesized is an attack on a major petrochemical production facility or petroleum refinery. To investigate potential health effects of such an attack, we have selected a high-volume (3 billion pounds, annual US production), volatile, aliphatic hydrocarbon, 1,3-butadiene (BD), as a prototype combustible organic compound. The characteristics of butadiene soot (BDS) that make it a useful model for large-scale organic combustion products are: 1) Freshly generated BDS particles are in the ultrafine range (>50% = 30-50 nm); 2) Polynuclear aromatic hydrocarbons (PAHs), including several carcinogens, are adsorbed to the surface of the particles and account for ~16% of the weight of freshly generated BDS; 3) Cultured human bronchial epithelial cells exposed to BDS contain the same spectrum of PAHs as BDS particles, display plasma membrane blebs, reduced proliferative capacity, and develop punctate fluorescence in lipid vesicles. Here, we demonstrate that the development of cellular fluorescence is time- and concentration-dependent. Cells exposed to a solution of sonicated BDS (1mg BDS/5ml medium) develop punctate fluorescence within 5 min. All cells fluoresce by 75 min. Fluorescence develops more slowly in cells exposed to dilutions of sonicated BDS (1:10-1:100). Transmission electron micrographs show that BDS-exposed cells have altered mitochondrial morphology and large cytoplasmic vesicles containing lamellar bodies. In BDS-exposed (5 and 50 mg/m3, 2 hr/day, 1-5 days) Balb/c mice, BDS particles aggregate within the bronchoalveolar space and remain there for at least 10 days after exposures end. Bronchoalveolar lavage fluid contained soot particles plus macrophages with phagocytosed soot particles. Thus, BDS can serve as a model respirable petrochemical combustion product causing deleterious effects to the respiratory system subsequent to acute inhalation exposure.

1055 TWO-WEEK INHALATION STUDIES WITH DISK-SHAPED PARTICLES OF POTASSIUM OCTATITANATE (TERRACESS TF) IN RATS

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Sponsor: S. Loveless.

Fibrous particles such as asbestos and some man-made fibers (MMF) have been known to produce carcinogenic or fibrogenic effects that are related to fiber dimensions, durability, biopersistance, and physicochemical characteristics. Disk-shaped particles of potassium octatitanate (Terracess TF) were manufactured in an effort to reduce toxicity that might result from the presence of fibers. A 2-week inhalation study was conducted to compare the toxicity of the disk-shaped material (TF) with that of previously tested fibrous potassium octatitanate (PT) fibers. Groups of 10 male rats were exposed whole-body to TF aerosols at concentrations of 0, 5, 25, or 100 mg/m3 for 6 hrs/day for 9 days over a two-week period. The mass median aerodynamic equivalent diameter of the aerosols was 2.4 to 2.9 µm. No adverse effects were observed in exposed rats in clinical observations, body weights, and clinical pathology including urine, hematology, and blood chemistry parameters. Lung and lung/brain weight ratio were not affected. No adverse histopathological effects were observed in the respiratory tract including nose, larynx/pharynx, trachea, and lungs. Inhaled particles were mostly phagocytized by free alveolar macrophages (AMs) in the alveolar air spaces. The alveolar walls enclosing particle-laden AMs maintained their normal structure indicating that disk-shaped TF particles have much lower toxicity than previously tested fibrous PT. Based on the lack of adverse effects in the respiratory tract, the no-observed-adverse-effect-level (NOAEL) for Terracess TF was considered to be 100 mg/m3 and the lung responses were comparable to that of "nuisance" type dusts at these concentrations.

1056 THE ACUTE EFFECTS OF TUNGSTEN ALLOYS (WA) ON THE RAT AIRWAY

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Penetrating munitions have traditionally been made with hard and heavy materials such as depleted uranium (DU). Because of the environmental and health concerns associated with DU, alternative materials including WA are now being developed and fielded. When WA munitions strike hard targets, aerosol clouds containing respirable particles as small as 0.2 µm are formed. Since military personnel near impact sites may be acutely exposed to high concentrations of aerosolized metals, the effects of WA on the airway were investigated. Male Sprague-Dawley rats were intratracheally instilled with either 20 mg of WA (91.1% W, 6.0% Ni, 2.9% Co) suspended in 250 µl of sterile saline or saline alone (control). After 24 hours (n=5/group), 48 hours (n=4/group) and 7 days (n=3/group), the animals were sacrificed; the lungs were lavaged with cold phosphate buffered saline and markers of inflammation and injury were measured in the lavage supernatant. Total protein (µg/ml) was significantly elevated in the WA exposed rats at 24hr (211.0+24.5) and 7 days (308.3+ 84.0) compared to controls (134.0+ 6.82 and 159.8+19.8, respectively). The WA treated animals also had significantly more lactate dehydrogenase 48hr (0.12+0.01 µg/ml) and 7 days (0.39+0.12 µg/ml) after exposure compared to controls (0.07+0.002 µg/ml at 48 hr and 0.06+0.01 µg/ml at 7 days). Similarly, the concentrations of β-glucuronidase were significantly elevated to 130.6+22.1 µg/ml at 24hr and 104.1+19.2 µg/ml at 7 days following WA exposure compared to controls (89.7+9.4 µg/ml at 24hr and 64.6+8.6 µg/ml at 7 days). Significant amounts of tungsten (121.1+24.5 ppm), nickel (8.4+1.4 ppm) and cobalt (1.3+0.2 ppm) were still present in the lung tissue 7 days after WA exposure compared to controls (0.02+0.002 ppm (W); below limit of detection (Ni) and (Co)). The detection of inflammation and metals in the lungs up to 7 days after WA exposure suggests that inhalation of munition particles may have not only acute but also long-term pulmonary effects.

1057 ACUTE EFFECT OF STAINLESS STEEL WELDING FUME INHALATION ON LUNG INJURY, INFLAMMATION, AND DEFENSE RESPONSES

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Many welders have experienced bronchitis, metal fume fever, lung function changes, and an increase in the incidence of lung infection. Questions remain unanswered regarding the causality and possible underlying mechanisms associated with the potential pulmonary effects of welding fume exposure. The objective was to assess the acute effect of stainless steel (SS) welding fume inhalation on lung injury, inflammation, and defense responses in rats. Male Sprague-Dawley rats were exposed to gas metal arc-SS welding fume at a concentration of 15 or 40 mg/m^3 x 3 hr/day x 10 days. The control group was exposed to filtered air. To assess lung defense responses, some animals in each group were intratracheally inoculated with 5 x 10^3 Listeria monocytogenes one day after the last daily exposure. Welding particles were collected during exposure, and elemental composition and particle size were determined. After exposure, parameters of lung injury, (lactate dehydrogenase and albumin) and inflammation (PMN influx) were measured in the bronchoalveolar lavage fluid recovered from each animal. In addition, particle-induced effects on pulmonary clearance of bacteria and macrophage function were assessed. The welding particles were comprised of (from highest to lowest concentration) Fe, Cr,

Mn, and Ni. Particle size distribution analysis indicated the mass median aerodynamic diameter to be 0.24 μm. Lactate dehydrogenase and albumin were significantly elevated ($p<0.05$) in the SS group at both doses compared to air controls. Interestingly, less than 10% of the cells recovered from the lungs of the SS group were PMNs. Lung bacteria clearance and macrophage production of reactive oxidants were significantly reduced ($p<0.05$) in the SS group. In summary, acute exposure of rats to SS welding fume caused significant lung damage, suppressed lung defense responses to bacterial infection, but had little effect on pulmonary inflammation. Additional chronic inhalation studies are needed to further examine the lung effects associated with SS welding fume exposure.

1058 SOLUBLE CHROMIUM IN WELDING FUME INCREASES SUSCEPTIBILITY TO PULMONARY BACTERIAL INFECTION IN RATS

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Frequency, duration, and severity of pulmonary infections have been shown to be increased in full-time welders. Animal studies have shown that manual metal arc, stainless steel welding fume (MMA-SS) increased susceptibility to lung infections. MMA-SS is primarily composed of iron (Fe), chromium (Cr), and nickel (Ni). The objective of this study was to determine which component of MMA-SS may alter lung defense. At day 0, male Sprague-Dawley rats were intratracheally instilled with MMA-SS at a concentration of 2 mg per rat or saline (vehicle control), or the metal constituents Fe_2O_3 (insoluble, 0.82 mg), NiO (insoluble, 0.06 mg), $Cr_2Na_2O_7$ (soluble, 0.60 mg) at the concentration at which they are present in the dose of MMA-SS. Another group of rats received a mixture of the three metals. At day 3, rats were intratracheally inoculated with 5×10^3 *Listeria monocytogenes*. At days 6, 8 and 10, left lungs were homogenized, cultured overnight, and colony-forming units counted to assess pulmonary bacterial clearance. At day 3 (prior to infection) and at days 6, 8 and 10, right lungs were lavaged to recover cells and fluid from the airspace. Cell differentials were performed and the production of reactive oxygen species by phagocytes was measured. Lactate dehydrogenase and albumin levels were measured in lavage fluid as indicators of lung damage. Exposure to MMA-SS, the soluble Cr, or the mixture of metals before infection significantly slowed the pulmonary clearance of the bacteria and increased lung tissue damage when compared to control, and animals treated with NiO or Fe_2O_3 did not differ from control. Animals pre-treated with soluble Cr or the mixture of all three metals had increased cell numbers of macrophages, neutrophils, and eosinophils, and oxidant production by phagocytes was increased at all time points when compared with the saline group. The results of this study indicate that the soluble Cr present in MMA-SS is likely to be the primary component responsible for the suppression of lung defense in rats.

1059 COMPARATIVE INFLAMMATORY LUNG RESPONSE IN A/J AND C57BL/6J MICE EXPOSED TO STAINLESS STEEL WELDING FUME

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Several epidemiology studies suggest that inhalation of welding fume (WF) increases lung cancer risk in welders. Stainless steel WF in particular contains both chromium and nickel, two known human carcinogens. However, controlled animal studies are undoubtedly needed to conclusively link WF exposure to increased lung cancer risk. Thus, we initiated a multipart study to compare the inflammatory and tumorigenic responses to WF in a lung tumor susceptible (A/J) and resistant (C57BL/6J) mouse strain. Mice were exposed by pharyngeal aspiration to a total of four doses, one every three days, of 5mg/kg manual metal arc-stainless steel WF (MMA-SS), 1.5 mg/kg soluble chromium (S-Cr), or saline vehicle. Bronchoalveolar lavage (BAL) was performed postmortem at one and four weeks after the final dose. Indices of lung cytotoxicity (lactate dehydrogenase release) and air-blood barrier damage (albumin) were measured in the acellular BAL fluid. The cellular BAL fraction was used to assess inflammation via polymorphonuclear leukocyte (PMN) infiltration. One week post-exposure, MMA-SS WF caused a slightly greater lung cytotoxicity compared to S-Cr, which was more pronounced in A/J versus the C57BL/6J mice. MMA-SS WF, but not S-Cr, caused greater air-blood barrier damage in A/J versus C57BL/6J mice. PMN infiltration was significantly elevated compared to control in both mouse strains one-week post-exposure to MMA-SS WF. S-Cr elicited a significant PMN response in A/J mice only at one week post-exposure. By four weeks post-exposure to MMA-SS WF or S-Cr, lung injury in both mouse strains returned to control. PMN infiltration decreased, but remained elevated in both strains exposed to MMA-SS WF, with the A/J mice showing greater inflammation. In conclusion, exposure to MMA-SS WF or S-Cr elicited greater lung injury and inflammation at one versus four weeks in both mouse strains. The A/J strain showed increased susceptibility to lung injury and inflammation compared to the C57BL/6J mice following exposure to MMA-SS WF or S-Cr.

1060 EXAMINING THE INFLAMMATORY RESPONSES OF HAPS: THE ROLE OF OZONE AND OTHER PHOTOCHEMICAL TRANSFORMATION PRODUCTS

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The chemistry and health effects of individual hazardous air pollutants (HAPS) have been studied for many years. Once released into the atmosphere, HAPS interact with hydroxyl radicals and ozone (created by photochemical processes), to produce many different products, whose toxic potential is currently unclear. In this study, three common HAPS (methanol, isoprene(ISO) and 1,3-butadiene(BD)) underwent photochemical transformations using real sunlight, generating a range of photochemical transformation products, including organic carbonyls such as formaldehyde and ozone. The objective of this study is to determine the role of ozone in the effects caused by the photochemically active HAPS mixtures. Using the UNC outdoor smog chambers interfaced with an in vitro exposure system, A549 cells were exposed to dynamic atmospheric mixtures. Exposure to the photochemically generated products of BD or ISO significantly increased cytotoxicity and cytokine gene expression compared to their injected primary photochemical transformation products, such as acrolein, formaldehyde and ozone for BD and methacrolein, methyl vinyl ketone, and ozone for ISO. Interestingly, exposure to the equivalent levels of ozone generated during the photochemical transformation of BD or ISO did not induce the same level of inflammatory cytokine release, suggesting that ozone alone is not the sole inducer of inflammatory responses in this system. However, for the photochemical transformation of methanol, generating primarily ozone and formaldehyde, ozone was the main inducer for both inflammation and cytotoxicity. Taken together these results indicate, that unlike simplistic atmospheric models such as methanol, ozone does not significantly account for the effects seen in more complex atmospheric mixtures, such as those generated by BD and ISO, and therefore full photochemical transformations and interactions must be carefully evaluated when investigating adverse health effects induced by exposure to HAPS.

1061 A GENETIC BASIS FOR INCREASED SENSITIVITY OF THE NEONATAL MOUSE LUNG TO OZONE

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Formed by the action of sunlight on nitrogen oxides and reactive hydrocarbons, ozone (O^3) is a powerful oxidant and respiratory irritant that leads to airway inflammation and pulmonary dysfunction. Studies have not established whether children are susceptible to ozone, even though animal studies have shown that neonates are more sensitive than adult rodents. Although it is postulated that children's potential for heightened susceptibility results from an underdeveloped respiratory system coupled with higher outdoor activity, the biological mechanisms underlying the predisposition of children to pollution-induced adverse pulmonary effects are unknown. Using a murine model, we tested the hypothesis that there is a genetic basis for the differential response of neonatal and adult lungs to inhaled pollutants. In this study, we exposed male and female adult and neonatal mice (15 to 16 d old) to 0.8ppm O^3 for 5 h from 8 inbred mouse strains. To insure that differences were due to biological responses, and not dose, mice from sensitive and resistant strains were exposed to ozone generated with ^{18}O and the lung burden of ^{18}O was determined. Inflammation and pulmonary injury were evaluated in lung lavage fluid recovered 24-hours post-exposure. Clear inter-strain differences in response to ozone, independent of dose, were seen in neonatal mice: SJL/J, C3H/HeJ, and Balb/cJ mice being the most sensitive while A/J, AKR/J, and 129x1/SvJ mice were the most resistant. Also, the neonatal response was greater than that observed in O^3-exposed adult mice, particularly in the SJL/J and C3H/HeJ strains. These results strongly suggest that genetic determinants do play an important role in the enhanced sensitivity of the young mammalian lung to ambient air pollutants. Further research will enable us to determine which genetic factors contribute to the heightened susceptibility of the juvenile lung to ozone, and to quantify the relative contribution of genes vs. the environment in the adverse effects of inhaled ozone.

1062 DISEASE-SPECIFIC SUSCEPTIBILITY TO ACUTE OZONE-INDUCED INJURY AND INFLAMMATION IN EIGHT RAT STRAINS

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Susceptibility to environmental pollutant-induced injuries may be influenced by presence of disease and genetic make-up. To identify disease-specific susceptibility phenotype, we used eight rat strains with or without genetic cardiovascular disease.

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster discussion, workshop, and poster sessions of the 45th Annual Meeting of the Society of Toxicology, held at the San Diego Convention Center, San Diego, March 5–9, 2006.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 500.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 534.

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