1042 RESPONSES OF LUNG PARENCHYMA TO CARBON NANOTUBES.

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An increasing number of applications have been and are being developed for new carbon allotropes such as carbon nanotubes (CNT). Pharyngeal aspiration by C57BL/6 mice was used to determine the pulmonary toxicity of CNT. Morphometry of paraffin sections from fixed lung tissue was used to determine the size of deposited CNT and the size of granulomatous lesions produced in response to the aspiration. Measurement of the Sirius red staining in sections was used to assess the connective tissue response. Lung responses were studied at 1 day, 7 days, 1 month and 2 months after a single CNT exposure of 0, 10, 20 or 40 μg/mouse. Examination of lung sections 1 day after aspiration, demonstrated that deposition of the CNT mass was generally in the first or second alveolar ducts proximal to the terminal bronchiole with an average diameter of 15.2±0.6 μm (mean±SE, n=12). At 1 day, CNT deposits were infiltrated with alveolar macrophages. At 7 days significant connective tissue accumulation was apparent within the CNT deposits. At 1 and 2 months, the granulomatous masses were encased in cuboidal epithelial cells. At 2 months, the granulomatous lesions accounted for 0, 0.7±0.1, 2.4±0.2 and 4.6±0.6 % of the alveolar parenchyma at doses of 0, 10, 20 and 40 μg/mouse, respectively. In addition to the granulomatous lesions there were also changes in the alveolar walls. For instance, the average thickness of Sirius red stained connective tissue in alveolar regions, excluding the granulomatous areas, was 0.10±.03, 0.20±.09, 0.3±0.09 and 0.5±0.1 μm at doses of 0, 10, 20 and 40 μg/mouse, respectively. The results demonstrate that CNT produce a rapid response in the alveolar region with both focal granulomatous lesions and a more generalized fibrotic response that is dose dependent.

1043 PULMONARY TOXICITY SCREENING STUDIES WITH NANO VS. FINE-SIZED QUARTZ AND TIO2 PARTICLES IN RATS.

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For most low solubility dusts such as titanium dioxide, the limited database suggests that fine-sized particles (e.g. pigment-grade) are less toxic than nano-sized particles of the same chemical composition. Fine-sized Min-U-Sil quartz particles are known to be extreme pulmonary toxicants and are classified as IARC Category 1 carcinogens, i.e., human carcinogens. Thus, is it the case that nanoquartz particles could be even more potent than fine-sized quartz particles? This study was designed as a preliminary screen to test 1) the nanoparticle vs. fine-size hypothesis of pulmonary toxicity. In this regard, we assessed whether the Min-U-Sil quartz particles, a known cytotoxic dust, impart significantly greater pulmonary toxicity in the lungs of rats when compared to nano-size quartz particles. In the first experiment with quartz, fine sized quartz (Min-U-Sil) (average diameter = 1.6 um) particles or nanoscale quartz particles (mean particle size = 50 nm) were instilled into the lungs of rats at doses of 1 or 5 mg/kg. Postexposure evaluations of bronchoalveolar lavage fluids were conducted at 1 day, 1 week, 1 and 3 months postexposure. Exposures to the Min-U-Sil quartz particles produced a significantly greater pulmonary inflammatory response. However, in a second experiment, following exposures to 1 or 5 mg/kg of Min-U-Sil quartz, another nanoquartz sample (10 nm) or fine-sized, submicron quartz particles (400 nm), the results suggested that the order of inflammatory potency was nano>Min-U-Sil>fine-sized quartz particles. In a third pulmonary bioassay study, rats were exposed to fine-sized TiO2 particles, TiO2 nanorods, and TiO2 nanodots at 1 or 5 mg/kg with postexposure evaluations at 1 day, 1 week, 1 and 3 months. No significant differences were measured in the inflammatory responses among any of the groups at any postexposure time periods. Our interim results suggest that the pulmonary toxicity of each fine-sized and nano-sized particle-type needs to be evaluated on a case-by-case basis.

1044 DEVELOPMENT OF ANIMAL MODELS OF INHALATION FEVER USING FINE AND NANOPARTICLE ZINC OXIDE EXPOSURES.

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Occupational fume fever is characterized by a transient flu-like syndrome associated with the inhalation of freshly formed metal ultrafine particles (UFP), notably zinc oxide (ZnO). The aims of this study were to 1) develop an animal model of metal fume fever in rats using either fine-sized (i.e. > 100 nm) or nano (NP - < 100 nm) ZnO particles. These studies are designed to better define the conditions and properties of ZnO nanoparticles (NP) vs. fine-sized ZnO particles that may be associated with lung injury and fume fever. Successful development of animal models could provide important scientific insights into fever development. Rats were exposed by inhalation for 1 or 3 hours to aerosols of fine-sized zinc oxide particles at 25, 35, or 50 mg/m3. Following recovery periods of 24 hrs, 72 hrs or 1 week, the lungs of ZnO and sham-exposed rats were lavaged and cells and BAL fluids were measured for cellular indicators (i.e., inflammatory cells) or noncellular mediators of inflammation and cell injury (e.g. BAL fluid levels of LDH, microprotein, or alkaline phosphatase). Our preliminary results with fine-sized ZnO demonstrated transient, pulmonary inflammatory effects. In this regard, animals exposed for 1 hr to ZnO at 25 or 35 mg/m3 demonstrated no inflammatory and very mild inflammatory response, respectively. Rats exposed for 3 hours to ZnO particles at concentrations ranging from 25 to 50 mg/m3 demonstrated transient pulmonary inflammatory responses which were evident after 24 and 72 hours but returned to control levels by 1 week postexposure. In subsequent experiments, groups of rats were exposed for 1 or 3 hrs to nano zinc oxide particles (mean particle size = 65 nm) at 25 mg/m3 and evaluated at 24, 72 and 168 hrs postexposure. The transient inflammatory effects of nano ZnO particles were not significantly different from exposures to fine-sized ZnO particles. Studies are ongoing to conduct experiments in rats with nano ZnO particles for 1 and 3 hrs at 35 and 50 mg/m3. Thusfar, we have not observed differences between the pulmonary responses to nano or fine-sized ZnO particles.

1045 PULMONARY EXPOSURE TO CARBON NANOTUBES INDUCES VASCULAR TOXICITY.

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Cardiovascular diseases, which in majority of cases stem from atherosclerosis, continue to be the principal cause of death in the United States. In addition to personal factors like hyperlipidemia and obesity, some environmental factors including cigarette smoking and air pollution, have been associated with cardiovascular diseases. Engineered nanosized particles, such as carbon nanotubes (CNT), are new materials of emerging technological importance in different industries. The unique physical characteristics of these particles raise concerns that they may have not only pulmonary toxicity but also extra-pulmonary toxicity. In the present study, we hypothesized that CNT pulmonary exposure is associated with oxidative and inflammatory responses in the vascular system, which might be a prerequisite of atherogenesis. C57BL/6 mice were exposed to CNT in doses (0.5; 1; 2 mg/kg) by single intra-pharyngeal installation and the mice were sacrificed at different time points (1; 7; 28; 60 days) after the exposure (the experimental settings have been related to pulmonary toxicity). By extra long quantitative PCR of mitochondrial (mt) DNA, we found that CNT exposure induced a dose-dependent aortic mtDNA damage, an oxidative stress dependable parameter, at day 7, 28 and 60 after exposure. Furthermore, by real-time PCR, we demonstrated that the CNT-induced oxidative changes are accompanied by altered expression of inflammatory genes, including MCP-1 and VCAM-1, in the heart. These effects might be a direct result from CNT which penetrate to the circulation or an indirect result of the lung inflammation. The direct effects of CNT were evaluated *in vitro* in human aortic endothelial cells (HAEC). After 2 hours exposure to CNT, we observed an increase of MCP-1, VCAM-1 and IL-8 mRNA levels in HAECs. CNT also dose-dependently induced low density lipoproteins (LDL) oxidation in the presence of HAECs. In conclusion, CNT induces direct or indirect toxic effects which might be predisposing factors for atherogenesis.

1046 TOXICOGENETIC AND TOXICOGENOMIC ANALYSIS OF ALCOHOL-INDUCED LIVER INJURY.

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Severe steatohepatitis is a hallmark of continuous exposure to alcoholic beverages and a significant health concern due to the development of fibrosis, cirrhosis and hepatocellular carcinoma. The mode of action for alcohol-induced liver damage is thought to involve multiple cell types and mediators with reactive oxygen species and inflammatory cytokines playing the major role. Despite the measurable progress in alcohol research in liver, little is known about the genetic factors that may contribute to a large variability in susceptibility to liver disease in humans. The development of a mouse intragastric model of alcohol-induced liver injury, an excellent model for human disease, allowed a number of important discoveries with help from genetically engineered animals. Here, we tested the hypothesis that by combining a state-of-the-art *in vivo* model of liver toxicity, our prior knowledge of the mechanisms of alcohol-induced liver injury, novel genomic and toxicologic analyses with knowledge of the genetic diversity in mouse inbred strains, a liver toxicity susceptibility state may be defined. A panel of six mouse inbred strains (male

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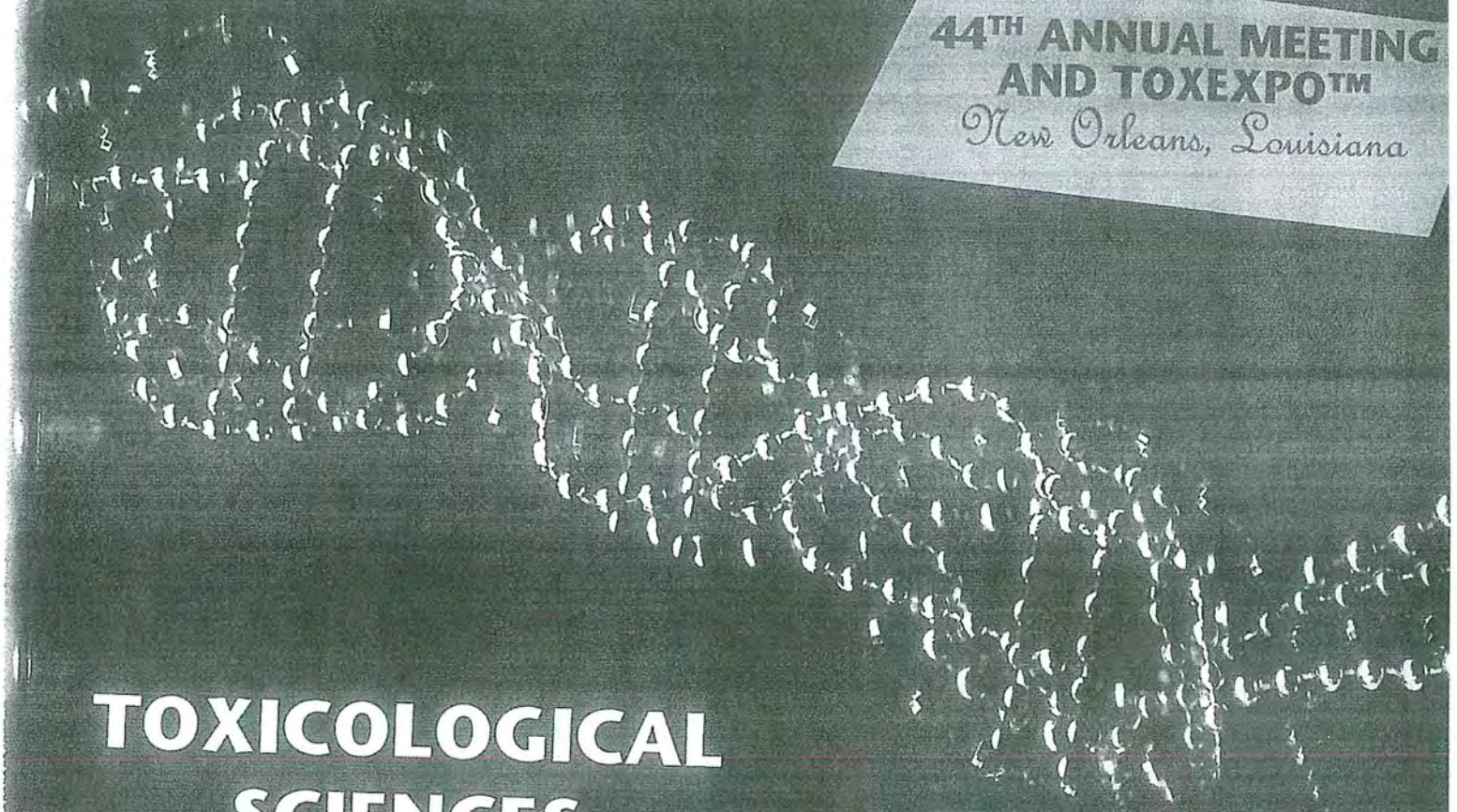

TOXICOLOGICAL SCIENCES

The Official Journal of the Society of Toxicology

Supplement

OXFORD UNIVERSITY PRESS

ISSN 1096-6080
Volume 84, Number S-1, March 2005

www.toxsci.oupjournals.org