mechanisms. Accordingly, to understand the role of these cytotoxic effects, we employed 2-chloroethyl ethyl sulfide (CEES), a monofunctional analog of SM. CEES exposure to mouse skin epidermal JB6 cells and dermal fibroblasts caused DNA-damage (phospho H2A.X and p53 and comet tail extent moment), oxidative stress and oxidative DNA damage (8-OHdG levels). In further studies distinguishing between oxidative and direct DNA-damaging effects of CEES, pretreatment with glutathione (GSH) or antioxidant trolox caused a decrease in CEES-induced oxidative stress and oxidative DNA damage. However, only GSH decreased CEES-induced total DNA damage, probably through formation of GSH-CEES conjugate detected by LC-MS analysis, indicating that oxidative stress may play a minor role in CEES-induced DNA damage. Unlike SM [forms both DNA interstrand cross links (ICL) and adducts], CEES can only form DNA adducts. Therefore, we further expanded our studies with nitrogen mustard (NM), a bifunctional analog of SM. Notably; SM studies in the laboratory setting are limited, as they require special facilities. DNA damage was assessed in JB6 cells via comet assay, which confirmed that NM acts as a strong DNA ICL forming agent. Trypan blue exclusion assay indicated that 0.75μM NM induced arrest in JB6 cell growth at 24h, suggesting a possible cell-cycle arrest, thereby allowing the cells to process DNA ICLs. An increase in comet length and phospho H2A.X and p53 at 16 and 24h following NM exposure also indicated DNA damage and the initiation of repair machineries. Studies are underway to understand the molecular mechanisms involved in repair of NM-induced DNA damage that can ultimately help us develop therapeutic strategies against NM- and SM-induced skin toxicity.

PL 1806 PULMONARY FIBROTIC RESPONSE FROM INHALED MULTIWALLED CARBON NANOTUBE EXPOSURE IN MICE.

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Inhalation exposure studies of mice were conducted with multiwalled carbon nanotubes (MWCNTs) to assess the fibrotic potential of this manufactured carbon nanomaterial. To address the hypothesis that MWCNTs cause persistent morphologic changes, male C57BL/6J mice were exposed in a whole-body inhalation system to a 5 mg/m^3 MWCNT aerosol for 5 hours/day for 12 days (4 times/week for 3 weeks). At the end of inhalation exposures, lungs were preserved by vascular perfusion of fixative while inflated with air at 1, 14, and 84 days post inhalation exposure. A separate, clean-air control group was also studied. Sections were prepared to analyze the distribution of lung burden following inhalation exposure. Morphometric measurements of Sirius Red staining were used to assess the connective tissue response. At day 1 post-exposure 86±4 and 14±6 percent of the lung burden (mean±SE, N=5) were in the alveolar and airway regions, respectively. Distribution within the alveolar region was 57±6, 7±5 and 20±4 percent in alveolar macrophages, alveolar airspaces and alveolar tissue, respectively. The mean linear intercept, a measure of the degree of alveolar expansion, was not significantly different between groups being 29.5±0.5, 29.6±0.6, 29.1 ±0.5 and 29.4±0.4 microns for clean-air controls, 1, 14 and 84 days MWCNT groups, respectively. The connective tissue in the alveolar region of MWCNT-exposed mice demonstrated a progressive increase in thickness over time (0.17±0.02, 0.22 ±0.02 and 0.26±0.03 for 1, 14 and 84 days) and was significantly different from clean-air controls (0.16±0.02) at 84 days. Despite the relatively low fraction of the lung burden being delivered to the alveolar tissue, the average thickness of connective tissue in the alveolar region increased by 53% in the 84 days after inhalation exposure. These results demonstrate that inhaled MWCNTs have the potential to produce a progressive, fibrotic response in the alveolar tissues of the lungs.

PL 1807 90-DAY INHALATION TOXICITY STUDY WITH CARBON NANOFIBERS IN RATS.

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VGCF™-H is a vapor grown carbon nanofiber with low acute inhalation toxicity; however the potential toxicity of repeated exposure is unknown. This subchronic inhalation toxicity study exposed groups of male and female rats to 0.54, (4.9 f/cc) 2.5 (56 f/cc), or 25 (252 f/cc) mg/m3 of VGCF™-H 6-hr/d, 5-d/wk over a 90-day period following OPPTS 840.3465 and OECD 413 guidelines. Groups of animals from the high and control group were allowed a 90-day recovery period to determine the reversibility of effects observed at the end of the exposure period. Exposure to VGCF™-H did not result in adverse changes in body weight or food consumption parameters nor were there any adverse clinical signs of toxicity in this study. No adverse effects were observed in the ophthalmological evaluations or in clinical pathology endpoints. Females exposed to 2.5 and male and females exposed to 25 mg/m3 demonstrated a significant increase in lung weights following exposure that was only partially reversed after the recovery period. Following the 2.5 and 25 mg/m3 exposure a subacute/chronic inflammation of the terminal bronchiole and alveolar duct areas of the lungs were noted wherein fiber-laden alveolar macrophages had accumulated. This finding was characterized by infiltrates of inflammatory cells and some thickening of interstitial walls and minimal to slight hypertrophy/hyperplasia of Type II epithelial cells for the 2.5 and 25 mg/m3 groups, respectively. Three months following exposure this inflammation was still present, but less severe. A non-specific inflammatory response was also noted in the nasal passages of exposed animals. Extrapulmonary fibers, occurring as single or very few fibers were sporadically noted in other organs; however no adverse pathological reactions were noted in the tissues containing these fibers. Therefore, the NOAEL for VGCF™-H nanofibers is considered to be 0.54 mg/m3 (4.9 fibers/cc) for male and female rats.

PL 1808 LINKING NANOMATERIAL PHYSICAL PARAMETERS TO TOXICITY: A SYSTEMATIC ASSESSMENT OF GOLD NANOMATERIALS.

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Gold nanomaterials (Au NMs) have been studied for their incorporation in biological applications due to their unique surface Plasmon resonance. Previous reports identified size and surface charge as critical in mediating the biological response, while other studies describe the importance of shape. However, within the nanotoxicology literature, comparisons of NMs are typically made across a variety of cell models using different biological assays. This study reports the evaluation of Au NMs using the HaCaT keratinocyte cell line and the same assays in order to provide a comprehensive assessment of Au NM physical parameters. Previously, surface charge was shown to mediate the mechanism of toxicity in Au spheres 1, and Au spheres were less toxic than rods2. In addition, altering the rod aspect ratio (AR) and surface chemistry impacted toxicity2,3. To further explore shape, this study evaluated nanocubes (NC: 50 nm), nanospheres (NS: 20, 50 nm), nanopills (NP: AR=2), and nanorods (NR: AR= 3) with a tannic acid surface using HaCaT cells. Typically, Au NRs coated with PEG display minimal cellular uptake. However, TEM imaging identified increased uptake in Au NP and NR when tannic acid was on the surface. Furthermore, cell viability was examined (MTS assay) at concentrations of 0, 10, 25, and 50 μg/ml for comparison with previously published data. The viability demonstrated a shape and concentration dependent response, with the ranking of toxicity: 20 nm NS < 60 nm NS < 50 nm NC < NP (AR=2) < NR (AR=3). Furthermore, changes in gene expression (25 μg/ml) demonstrated a shape dependent increase in stress response genes. This data in combination with previous data, illustrated that several physical parameters mediated Au NM toxicity in keratinocytes, emphasizing the link between NM characterization and toxicity.

PL 1809 THE ROLE OF IL-1β SIGNALING IN NICKEL ASSOCIATED MULTIWALLED CARBON NANOTUBE-INDUCED ACUTE PULMONARY EOSINOPHILIA.

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Exposure to certain engineered nanomaterials (ENM) has been associated with pathological changes in animal models raising concern that human health effects will emerge with increasing use of ENM, such as multiwalled carbon nanotubes (MWCNT). We had previously shown that a correlation exists between the amount of nickel associated with MWCNT and assembly of the NLRP3 inflammasome (NLRP3) resulting in conversation of pro-IL-1β to the active form. Furthermore, we have shown that NLRP3 activation in vitro correlates strongly with lung inflammation and pathology. In this study, we investigated the role of IL-1 receptor signaling in the induction of acute pulmonary eosinophilia. C57Bl/6 and IL-1 receptor null mice (IL-1R-/-) mice instilled with MWCNTs FA04 (2.54%Ni) and FA21 (5.54% Nickel) underwent whole lung lavage at 24 hours, 4, 7 and 14 days post-exposure. Differential cell counts, Eosinophil Peroxidase Assay (EPO), flow cytometry and histological evaluation of tissue sections were performed. Analysis of the results revealed that 24 hours of exposure to MWCNT FA21 was effective in inducing pulmonary inflammation as indicated by eosinophil influx into the airways of wild type mice. The cell differential count and EPO assay confirmed the presence of eosinophils in the airway of MWCNT exposed WT mice. The initial acute inflammatory response was diminished in mice deficient for the IL-1 receptor. However, in later time points, this inflammatory response was heightened in the lungs of IL-1R-/- mice, compared to the wild type mice. These data suggest an important role for IL-1β signaling in the regulation of the inflammatory response and a potential mechanism for the clearance of Ni-MWCNT from the lung. This work was supported by NIH grants NRSA F32 ES019816 RC2-ES018742 and P20-RR017670.

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