1496 COMPARATIVE PULMONARY TOXICITY STUDY OF 3 DIFFERENT DISPERSIONS OF NANO- TIO2 PARTICLES IN RATS.

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In order to evaluate the effects of size of poorly soluble, low-toxicity (PSLT) particles on pulmonary toxicity, not only the effects of the primary sizes of these particles but also the effects of their dispersion need to be examined.
The aim of this study was to assess the lung toxicity of 3 different, well-characterized dispersions of anatase nano-TiO2 particles in rats. The average secondary particle size of these TiO2 particles, which were prepared from the same primary-sized nanoparticles, was approximately 18, 65, and 300 nm. Groups of male Crl:CD (SD) rats were intratracheally instilled with 5 mg/kg of these nano-TiO2 particles dispersed in 0.2–1.3% of disodium phosphate (DSP) solution. DSP solution- and Min-U-Sil quartz-instilled rats served as vehicle and positive controls, respectively. Following the instillations, the bronchoalveolar lavage fluid of the rats was examined for inflammatory markers such as the number of white blood cells, LDH and protein. The histopathology of the lung, liver, kidney, spleen, and cerebrum at post-instillation timepoints of 24 hours, 3 days, 1 week, 4 weeks, and 3 months was also examined.
Mild inflammatory responses in the lung were observed in the 18 nm- and 300 nm-sized TiO2 particle-instilled groups at 24 hours, 3 days, and 1 week after the instillations. In contrast, in the 65 nm-sized TiO2 particle-instilled group, only minimal inflammatory responses were observed in the lung at all the timepoints. At 4 weeks after the instillation, the lung inflammatory responses were comparable among these 3 TiO2 groups and the vehicle control group. Thus, it was concluded that these TiO2 particle types produced only transient pulmonary inflammatory effects. In all the groups, no histopathological changes were observed in the liver, kidney, spleen, or cerebrum.

1497 INHALATION OF CARBON NANOTUBES INDUCES OXIDATIVE STRESS AND CYTOKINE RESPONSE CAUSING RESPIRATORY IMPAIRMENT AND PULMONARY FIBROSIS IN MICE.

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Due to their unique physico-chemical, electrical, mechanical and thermal properties, single walled carbon nanotubes (SWCNT) have numerous applications in electronics, aerospace devices, and computers, chemical, polymer and pharmaceutical industries. Previously, we have reported that pharyngeal exposure of C57BL/6 mice to SWCNT caused dose-dependent formation of granulomatous bronchial interstitial pneumonia, fibrosis, oxidative stress, acute inflammatory/cytokine responses and decrease in pulmonary function. In this study, we investigated whether whole-body inhalation exposure to aerosolized SWCNT (using generation system developed at NIOSH, Baron et al., 2007) caused pulmonary toxicity and early onset of fibrogenic response similar to adverse pulmonary toxicity reported previously. Here, we report that both inhalation and aspiration exposures to unrefined SWCNT (HiPco, CNI, Inc, TX) resulted in an augmentation of biomarkers of inflammation and oxidative stress as evidenced by ESR detection of POBN-spin trapped carbon centered radicals and significant depletion of antioxidants in mouse lungs, recruitment of inflammatory cells and increase in pro-inflammatory cytokines assessed in the bronchoalveolar lavage (BAL) fluid, and early onset of granulomatous lesions and interstitial pulmonary fibrosis. The chain of pathological events in both exposure routes was realized through synergized interactions of early inflammatory response and oxidative stress culminating in the development of multifocal granulomatous pneumonia and robust interstitial fibrosis.

1498 CYTOTOXICITY OF NANOSIZED TIO2 AND MULTI-WALLED CARBON NANOTUBES ON CELL PROLIFERATION *IN VITRO*.

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There is growing concern that as commercial development of nanosized materials develops there is a corresponding need for safety assessment of these materials. Recent studies have demonstrated granuloma formation, fibrosis and cytokine mediated inflammation associated with pulmonary exposure to a variety of nanosized materials. In this study TiO2 and Multiwalled carbon nanotubes (MWCN) were tested in 4 cell lines primarily associated with the lung: A549 human bronchial epithelial cells (hBEC)(ATCC # CCL-185), L2 rat bronchial epithelial cells (rBEC)(ATCC # CCL-149, rat alveolar macrophages(AM)(ATCC# CRL-2192) and CHOK1 cells. To test the relative toxicity of these materials each cell line was grown to confluence in appropriate media and treated for 48 hrs with serial dilutions of TiO2 and MWCN of 0.001 to 30µg/µl. 6 well dishes containing 1x10 5 cells/well were treated. TiO2 and MWCN were suspended in appropriate media and sonicated for 30 minutes prior to treatment. All plates grew for 48 hrs at 37o C and 5% CO2. Wells containing cells with no treatment served as negative controls while H2O2 served as the positive control. The concentration of the materials that suppressed the cell numbers by 50% in comparison to the untreated controls (IC50) was determined and used as an index for cytotoxic activity. Three independent assays were performed with each material in duplicate per concentration. The results were that the IC50s for the MWCN were 0.06, 0.5, 0.4, and 2.0µg/µl for AM, hBEC, rBEC and CHOK1, respectively. For TiO2 the IC50 values were 0.04, 0.3, 0.01 and 0.8 µg/µl for AM, hBEC, rBEC and CHOK1. Confocal microscopy showed extensive uptake of the nanomaterials by AM which may account for their greater sensitivity. Also, rBEC cells were very sensitive to TiO2 suggesting that the rat may not be an ideal model for human toxicity of this material. Funding for this study was provided by the HESI Nanomaterial Safety Committee.

1499 DIRECT FIBROGENIC EFFECTS OF DISPERSED SINGLE WALLED CARBON NANOTUBES ON HUMAN LUNG FIBROBLASTS.

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Nanomaterials, including single walled carbon nanotubes (SWCNT), are being developed for a variety of commercial products. However, toxicity of these new materials has not yet been identified due to limited information regarding the relationship between their unique physicochemical properties and potential toxicity. We reported recently that dispersed SWCNT (DSWCNT) exposure in mice caused a rapid and progressive interstitial lung fibrosis without a persistent inflammation. From these studies, it was hypothesized that DSWCNT, due to their exceptionally small size, can quickly penetrate the alveolar epithelial wall, enter the underlying tissue, and induce a "direct" fibrotic effect by interacting with cells at the site of particle accumulation to induce collagen deposition. The present study investigated the effects of *in vitro* DSWCNT exposure of human lung fibroblasts on proliferation and collagen production using cell multiplication (MTT), and Western blot techniques. DSWCNT were prepared using methods described previously in our laboratory. The results demonstrated that: 1) pulmonary exposure to DSWCNT (10 µg/mouse) did not cause significant lung injury; however, DSWCNT did penetrate into the interstitial lung tissue as analyzed by electron microscopy; 2) DSWCNT stimulated fibroblast cell growth *in vitro* at doses of 0.3-1 µg/ml; 3) DSWCNT also induced collagen production and collagenase (MMP9) activation in the treated fibroblasts. We also observed that the dispersion status or size of the SWCNT is a critical factor in determining fibrogenicity. These findings provide new insights into the mechanisms of SWCNT-induced lung fibrosis and may aid in developing models for risk assessment of these nanoparticles.

1500 PULMONARY RESPONSE TO INHALED NICKEL NANOPARTICLES.

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Nanotechnology is a rapidly growing field which utilizes nanosized materials with unique properties (i.e. biological). Presently, there are concerns about the potential health effects that these materials may cause. For this reason, it is important that research is conducted that addresses specific questions concerning the safety and hazards of these materials. An exploratory study was conducted to assess the pulmonary toxicity of acute exposure to nickel nanoparticles (Ni NPs) (CMD=50nm) in male C57BL/6 mice. Utilizing a whole-body exposure system, male mice inhaled either spark generated Ni NPs (GFG-100 Palas, Germany) or filtered air for 4hrs at the following concentrations: 1000, 150, or 33 µg/m3. Bronchoalveolar lavage fluid was collected 24 hours post-exposure to assess neutrophil infiltration and protein leakage into the lungs. A neutrophil population of 28%, 5%, and 2% was observed for 1000, 150, and 33 µg/m3 Ni NPs exposed mice, respectively. Compared to filtered air exposed mice, only the 1000 and 150 µg/m3 data were statistically significant. No difference in the protein levels was observed at any of the concentrations used. These results illustrate a dose-dependent inflammatory response to acute exposures of Ni NPs. Gene expression analyses using real time reverse transcription-polymerase chain reaction (RT2-PCR) showed increased 1L-6, HO-1,

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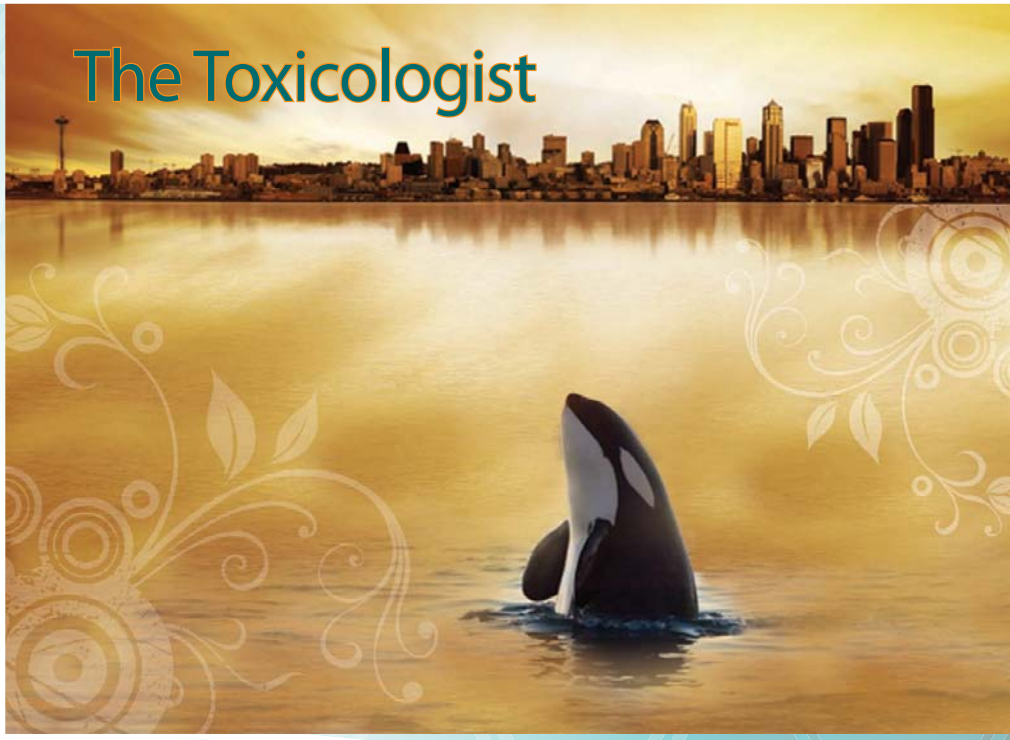

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