tissue. The concentration rank by lobe was: left>caudal>cranial>accessory>middle. At 1 DPE, both AgNPs had a diffuse pattern of silver AMG tissue staining, with heavy localization in macrophages. At 7 DPE, Ag localized to the epithelium in the bronchoalveolar duct junction (BADJ), in addition to macrophages. At 21 DPE, the Ag was found in the subepithelium of the bronchoalveolar duct junction with fewer stained macrophages observed. At 21 DPE similar amounts of Ag remained in the lung for 110nm and 20nm AgNPs (4.25μg/g and 3.64μg/g, respectively). We conclude that 1) the 110nm AgNPs dispersed more uniformly than the 20nm AgNPs between lung lobes, 2) the pattern of silver distribution indicates prolonged localization to the BADJ and 3) the lung retains approximately 25% of delivered dose at 21 DPE. Support:U01ES02027 and P42ES004699. AgNPs supplied by NIH NCNHIR consortium.

PS 829 Inhaled Nano-TiO2 Size Distribution along with Mass Concentration Define Lung Responses

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Currently, there is no consensus regarding the exposure metric that best expresses the nanoparticle (NP) dose. Although the surface area was extensively studied for the inflammatory reaction, it has not been validated for cytotoxicity or oxidative stress. Since inhaled NP deposit and interact with lung cells according to their agglomerates size, we hypothesized that the mass concentration combined to the aerosol's size distribution is suitable for NP risk assessment. The objective of this study was to evaluate different exposure metrics for inhaled 5 nm TiO2 aerosols composed of small (SA) (<100 nm) or large (LA) (>100 nm) agglomerates at 2, 7 and 20 mg/m3 on rat lungs inflammatory, cytotoxicity and oxidative stress responses. Six groups of male F344 rats (n=6) were nose-only exposed for 6 hr to the different 5 nm TiO2 aerosols. The control group was exposed to air. Exposures were characterized by weight measurement for mass concentration and with an electrical low pressure impactor for number concentration and size distribution. Bronchoalveolar lavages (BAL) were analyzed 16 hr after exposure. Our results showed that the 7 mg/m3 LA and the 20 mg/m3, both LA and SA aerosols, increased the number of neutrophils compared to controls. The 20 mg/m3 SA significantly ($p<0.05$) increased LDH activity and 8-isoprostane concentration compared to controls. When the size distribution of NP was considered with the mass as an exposure metric, we found a strong correlation ($r>0.97$) with the number of neutrophils for SA and LA. For LDH and 8-isoprostane, we observed a high correlation ($r>0.89$) only with SA. These data show that the mass concentration alone is not sufficient to adequately predict oxidant and cytotoxic pulmonary effects. Overall, our study indicates that considering the NP size distribution along with the mass concentration contribute to a more mechanistic discrimination of respiratory effects.

PS 830 Cell Transformation Potential of Nano-Cerium Oxide ($nCeO_2$), Nano-Ferric Oxide (nFe_2O_3) Compared to Multiwalled Carbon Nanotubes (MWCNT)

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Unique physicochemical properties of engineered nanomaterials (ENMs), including CNTs and metal oxides, are distinct from their micron-sized counterparts evidenced by their unique biological effects. Previous studies have suggested potential lung disorders induced by these EMNs. Recent animal study indicated cancer promotion by MWCNT. Our previous *in vitro* work showed subchronic single walled CNT or MWCNT exposure can cause human lung epithelial cell transformation which formed tumors when subcutaneously injected into mice. These findings raise ENM-induced carcinogenesis concerns due to rapid growth in ENM applications, including widely used $nCeO_2$, nFe_2O_3 and MWCNT. The present work tested cell transformation potential of $nCeO_2$ and nFe_2O_3 compared to MWCNT as a positive tumorigenic control. Primary human small airway epithelial cells were treated with a sub-toxic, low dose of ENMs (62.5 ng/cm^2 of $nCeO_2$ and nFe_2O_3, or 60 ng/cm^2 of MWCNT), with albumin and saline as dispersant and no treatment controls, respectively. Cells were continuously exposed to ENM containing medium for 6 weeks. Following exposure, cells were assayed for cell transformation and cancer hallmark analysis. Increased cell proliferation suggested significant cell stimulation by $nCeO_2$ and MWCNT. Next, nFe_2O_3 and MWCNT-treated cells formed soft agar colonies and significantly enhanced cell invasion which suggests their potential cell transformative effect. These results will guide future *in vivo* studies to confirm the observation and to assess the relevancy of our subchronic *in vitro* exposure model. Such an *in vitro* model may serve as a simple/fast/high throughput tool to screen countless ENM to predict cell transformation/carcinogenic potential and addresses a critical need in ENM risk assessment.
Disclaimer: Presented findings and conclusions are the authors and do not necessarily represent those of the National Institute for Occupational Safety and Health.

PS 831 Evaluation of Pulmonary Response to Tungsten Oxide (WO_3) Nanoparticles in Golden Syrian Hamsters

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Tungsten oxide (WO_3) nanoparticles possess excellent photochromic, electrochromic, thermochromic and catalytic properties. Extensive industrial and military uses have raised the possibilities of human occupational and environmental exposure with concomitant health concerns. To evaluate the effects of such particles in the lung, Golden Syrian Hamsters were exposed by inhalation to aerosols of WO_3 nanoparticles (Nanostructured and Amorphous Materials, Inc., TX). Hamsters were divided into four groups – a control group that was exposed to distilled water and 3 treatment groups that were exposed to 5, 10 or 20 mg/m^3 WO_3 nanoparticles (4 hrs/day, for 4 days). Animals were euthanized 24 hours post-exposure and bronchoalveolar lavage fluid (BALF) was collected. Lungs were then fixed by insufflations of 10% formalin. Tissue sections were analyzed using bright and dark field microscopy. The average size of WO_3 nanoparticles, 71 ±34 nm, was confirmed using TEM. There was a significant increase ($p<0.05$) in total cell counts 22.3 ±0.1, 21.6 ±2.5, and 29.3 ±0.1 (x10^4 cells/ml) of BALF from treated groups (5, 10 or 20 mg/m^3 respectively), as compared to controls (15.6 ±1.4 x 10^4 cells/ml). BALF protein levels increased in treated groups (106.1, 110.7 and 124.1 μg/ml) as compared to controls (98.9 μg/ml). The number of macrophages increased in the BALF treated group (76.8, 65.6 and 145.5 cells/field at X400) as compared to controls (29.2 cells/field at X400) without increase in the number of PMN's. Dark field micrographs of tissue sections revealed WO_3 nanoparticles present in alveolar macrophages that were dispersed in large numbers throughout the lung. Nanoparticles were also identified on airway epithelium, within airway epithelial cells and in interstitial areas of alveolar structures. Results from these experiments indicate that WO_3 nanoparticles can reach alveolar space, enter epithelial layers and penetrate to interstitial structures.

PS 832 Evaluating Metallic NP-Induced Stress Using a Neuronal Coculture Model

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To date nanotoxicity studies on the central nervous system are limited and conflicting with discrepancies between animal models and humans adding to the complexity. A previous study focused on establishing a neuronal co-culture model which included neurons and their microglia, to evaluate the phagocytic response effects of nanoparticles (NPs). As a continuation, the present study examined the toxicity of different nanometals which included silver (Ag) and gold (Au) as well as known neurotoxicants manganese (Mn), aluminum (Al), and copper (Cu); and while all of the metallic NPs had an initial size of 80 nm, dispersion resulted in varying degrees of aggregation. The cells were dosed with realistic exposure levels of the different metallic NPs to represent a daily or weekly exposure based on the current limits reported for the bulk metals by OSHA. In addition to viability and reactive oxygen species (ROS) production, key stress genes (NF-κB, TNFα, IL-6) were selected to examine changes following varying times of exposure (4, 8, and 24 h) to metallic NP. The Mn-NPs induced significant toxicity at daily and weekly exposure levels, while Cu-NP showed toxicity only at the weekly exposure level. Furthermore, the Mn-NP treatment demonstrated concentration dependent increases in ROS production and TNFα and IL-6 expression over time while NFκB remained unchanged. Surprisingly, while the Cu-NP treatment induced toxicity, and increased ROS production, the stress response profiles showed no changes at 4 h, and d decreased expression for IL-6 and TNF-α following 8 and 24 h. In comparison, the Ag-, Au-, and Al-NP treatments did not induce toxicity or significant increases in ROS and showed no changes in gene expression at either treatment concentration or time point. Taken together, the results demonstrated that the co-culture model provides enhanced sensitivity for studying NP induced stress responses and the nanometals displayed differential toxicity with Mn>Cu>Al/Ag/Au.

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