2187 *In Vitro* Intestinal Toxicity of Multiwalled Carbon Nanotubes

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Multi-walled carbon nanotubes (MWCNTs) are emerging nanomaterials that are widely used in industrial, engineering, biological and medicinal applications due to their unique physiochemical, optical and electrical properties. The release of these nanomaterials into the environment poses a potential for human exposure following inhalation and ingestion. The purpose of this study was to assess the *in vitro* toxicity of four MWCNTs with outside diameter of <8 nm, 13-18 nm, 20-30 nm, and >50 nm and two functionalized MWCNTs (-OH, -COOH) with outside diameter 20-30 nm in a rat intestinal cell model (IEC-6 cells). Pluronic (0.1%), a non-ionic surfactant was used to stabilize MWCNT dispersion. Hydrodynamic diameter and zeta potential were calculated to determine the sonication time at which the MWCNTs were optimally dispersed. Based on the results, the MWCNTs suspended in Dulbecco's media and 0.1% pluronic were probe sonicated for 15 min before dosing. IEC-6 cells were plated in 96-well plate with 60K cells/well for 24 h before dosing. Media with 10% fetal bovine serum was the negative control and Triton X-100 (0.3%) was the positive control. Cells were then exposed to the MWCNTs at various concentrations (0.3-300 µg/ml) for 24 h. Following incubation, the cells were washed with media and cytotoxicity was assessed using a colorimetric method that measures mitochondrial activity. Only the <8 nm MWCNT displayed cytotoxic effects, inducing 50% cell death at a concentration of 300 ug/ml. All other MWCNTs tested were negative. The results suggest that the outside diameter of MWCNTs is an important factor in the cytotoxicity of these nanomaterials in rat IEC-6 cells. *This abstract does not necessarily represent US EPA policy.*

2188 Differential Mitochondrial Perturbations among Primary, Cancerous, and Asthmatic Lung Cell-Types after Exposure to Engineered Nanomaterials

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The use of metallic nanoparticles as additives in consumer and industrial products is rapidly increasing. Specifically, zinc and aluminum are highly utilized as fuel additives and other automotive applications, thus increasing the risk of occupational, consumer, and environmental exposure to these engineered nanomaterials. Zinc (Zn) and aluminum (Al) have been shown to accumulate within mitochondria and perturb mitochondrial processes, however the specific mechanisms of toxic action remain unknown. There is a need to develop toxicological testing paradigms focused on the elucidation of Zn, ZnO, Al, and Al_2O_3 toxicities. The purpose of this study was to determine the effect of zinc and aluminum nanoparticles, as well as their oxide counterparts, on human mitochondrial health along the electron transport chain (ETC). To determine changes in mitochondrial health, three different epithelial cell-types from the upper airway with varying phenotypes were selected as a test system. The three phenotypes include primary cells (PTBE), cancer cells (A549), and asthma cells (DHBE). These cells were selected to represent a healthy human population as well as two unique sensitive subpopulations. Our hypothesis is that the differential baseline mitochondrial health profiles will be key determinants of induced nanotoxicity. Specifically, the primary and asthma cells are more sensitive to the metal nanoparticle exposures when compared to the cancer cell-type because cancer cells as less susceptible to ETC perturbations through oxidative stress. The mitochondria in each cell-type was characterized before and after exposure to normalize mitochondrial health metrics. Characterization included, mitochondrial morphology, mitochondrial dehydrogenase activity, and gene/protein expression. Differential dose-response patterns in mitochondrial activity were seen in mitochondrial morphology assessments, degradation of cristae structure, decreased mitochondrial dehydrogenase activity, and increased antioxidant response. While all cell-types exhibited changes in mitochondrial health, the primary cells showed the most pronounced alteration of mitochondrial structures, while the asthma cell-type experienced increased proinflammatory responses. The changes in the mitochondrial structure and function in the cancer derived cell-type were minor compared to those observed in the other cells. These results give newfound insight that will influence safety testing guidelines by providing evidence to use more realistic healthy (PTBE) or compromised (DBHE) populations.

2189 Assessing Organomodified Nanoclay Pulmonary Toxicity across Its Life Cycle Using Integrated Exposure and *In Vitro*/*In Vivo* Approaches

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Organomodified nanoclay (ONC)-enabled composite technology continues to revolutionize commercial, industrial, and consumer materials, which entail unique coatings, thin film, and durable polymer applications. Pulmonary health risks to ONCs along their chemical life cycle, however, are not understood. This project aims to link physicochemical properties of ONCs in their as-produced, product breakdown, and incinerated byproduct forms to adverse pulmonary effects, with crystalline silica (CS) as a comparative benchmark particle. Low dose pre-incinerated ONC exposure (Clois30B, Clois25A, and to lesser extent, Clois93A) caused a mixed Th1/Th2/Th17 inflammation response while higher doses elicited a mixed apoptotic/necrotic effect in human macrophages. Uncoated CloisNa caused membrane damage, inflammasome activation, and apoptosis in epithelial and macrophage cells, with minimal response observed in incinerated ONC-exposed cells. High dose incinerated uncoated and ONC caused elevated IL-8 and MCP-1 release in both *in vitro* and *in vivo* models, and were greater than CS. Both CloisNa and Clois30B stimulated *in vitro* fibroblast proliferation and collagen production. Similar doses of pre- and post-incinerated Clois30B in a C57BL/6 mouse model caused a delayed, low-grade inflammatory response followed by an increased pro-fibrotic and inflammatory cytokine profile. Finally, dust release was compared across scenarios using different base sandpaper material and grits on breakdown of synthesized nanoclay-enabled polypropylene composite (NPC). Coarse grit sandpaper on 4% Clois93A NPC, a composite with well-dispersed nanoclay, caused the largest release of respirable particles compared to 4% Clois25A NPC, virgin polypropylene, all 1% NPCs, and all composites sanded with fine grit sandpaper. These findings suggest 1) coating presence, type, and incineration status determine pulmonary inflammation and fibrotic signaling, and 2) sandpaper characteristics, different ONC coatings, and percent ONC inclusion affected NPC breakdown and airborne particle mass and size distributions. Evaluation of ONCs along their life cycle, by incorporating exposure estimates into current *in vitro/in vivo* comparative toxicological tiered frameworks, provides key information for prevention-by-design and material user approaches.

2190 Biomimetic *In Vitro/In Vivo* Models for Assessment of Hazardous Pulmonary Effects of Nanoparticles

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Potential human exposure to respirable nanoparticles (NPs) has become a major concern with increasing evidence showing that NP pulmonary exposure results in particle deposition in deep lung tissues and causes pathological changes. Such adverse health effects have not been well assessed partially because it is impossible to assess the toxicities of countless NPs using animal models, and limited relevance of *in vitro* models to evaluate specific toxicities of NPs. To address the challenges, we have developed multiple *in vitro* models to assess the biological and toxicological activities of well-characterized NPs with the identification of target lung cells of pulmonary exposed NPs from animal studies. Based on established *in vivo* doses that induce significant pulmonary disorders, physiologically relevant *in vitro* doses (i.e., 0.02 - 0.2 µg/cm^2) of NPs, including carbon nanotubes (CNTs) and iron oxide NPs (nFe_2O_3), were used to evaluate their toxic effects on human lung cells under long-term exposure condition (up to 6.5 months). Present study data showed that NPs were able to induce dose- and time-dependent cytotoxicity, inflammation, fibrogenesis and neoplastic transformation of human lung cells, consistent with *in vivo* data. Our *in vitro* studies determined specific particle type- and cell type-dependent NP-induced cell proliferation, anchorage-independent growth, apoptosis evasion, and increased cell migration and invasion. Furthermore, by developing a fibroblast stem cells (FSC)-enriched fibroblast focus model to mimic *in vivo* fibrogenic response, we demonstrated a dose-dependent increase in fibroblast focus formation and collagen production by primary lung fibroblasts treated with multi-walled carbon nanotubes (MWCNTs). This result unveils a novel mechanism of nanotube-induced fibrogenesis through ALDH-dependent FSC activation. The described *in vivo-in vitro* combination approach will support the utility of *in vitro* models as rapid screening and predictive tools for risk assessment of nanomaterials.

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