2782 An Evaluation of Genotoxicity from As-Produced and Post-Production Modification of Multi-Walled Carbon Nanotubes

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Companies synthesize or purchase bulk as-produced multi-walled carbon nanotubes (AP-MW) and apply coatings such as polymers (PC-MW) or aluminum oxide (AL-MW). The aim of this study was to measure the genotoxic response of three AP-MW (1, 2 & 3) and their coated counterparts (PC-MW 1 & 2 and AL-MW 3) to determine the effect of coating. Genotoxicity was measured via micronuclei assay with pancentromere staining and two-color cell cycle analysis. Pancentromere staining determined whether the DNA damage is clastogenic (chromosome breakage) or aneugenic (whole chromosome gain or loss). Immortalized human lung epithelial cells, BEAS-2B, were exposed to all MW for 24 hours. Significant necrosis was measured for all but AP-MW1 at the highest dose, 24 µg/cm2. Exposure to the lowest and most occupationally-relevant dose, 0.024 µg/cm2, of AL-MW was also significantly necrotic. Tunel assay of cells exposed to 2.4 µg/cm2 MW for 24 hours showed no evidence of apoptosis. All but PC-MW2 produced increased amounts of micronuclei after exposure to 24 µg/cm2 for 24 hours; AP-MW2 and AP-MW3 had greater micronuclei than their coated counterparts. All MW produced increased amounts of micronuclei after exposure to 2.4 µg/cm2 for 24 hours, however differences from the effect of coating cannot be detected at this time. Pancentromere staining indicated AL-MW to be a clastogen at the 2.4 µg/cm2 dose, while all other MW induced a combination of clastogenic and aneugenic effects. Preliminary two-color cell cycle analysis showed no evidence of cell cycle disruption after 24 hour treatment to 2.4 µg/cm2 MW. In order to determine the effect of coating on the genotoxic response, further analysis of the mitotic spindle and separation of genetic material leading to aneuploidy is required. However, this preliminary evaluation indicates that human lung epithelial cells exposed to AP-MW and their coated counterparts produces a genotoxic response.

2783 Nano-Ferric Oxide Induced Neoplastic-Like Transformation in a Human Primary Cell Model: Iron Homeostasis Disruption?

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As incorporation of engineered nanomaterials (ENMs) into new technologies rise, the potential for long-term, low dose inhalation exposures is expected with largely unknown adverse outcomes to human health. Past ENM toxicity assessment has focused on acute and relatively short term sub-chronic exposures associated with inflammation and fibrosis, with relatively little attention paid to ENM-associated tumorigenesis. The current study evaluated the use of a human primary small airway epithelial cell (pSAEC) model to serve as a Tier I neoplastic transformation screening model for proposed ENM tiered risk assessment. Low passage pSAECs were continuously exposed *in vitro* to 0.6 µg/cm2 of nano-sized cerium oxide (CeO2) or ferric oxide (Fe2O3) for 6 and 10 weeks. Multi-walled carbon nanotube (MWCNT Mitsui 7; 0.06 µg/cm2), with known transformation and lung cancer promotion potential, served as a positive control while saline or dispersant exposed cells served as passage controls. At each time point, exposed cells were evaluated for several cancer hallmarks to evaluate neoplastic transformation potential. At 10 weeks, Fe2O3-exposed cells displayed significant enhanced proliferation, invasion, soft agar colony formation and colony forming unit ability suggesting a neoplastic-like transformation. MWCNT-exposed cells exhibited increased colony formation ability while nCeO2 was negative in all assays except proliferation. Next, Fe2O3 and MWCNT soft agar colonies were isolated and placed in culture to evaluate persistence and longevity of the transformed phenotype. All isolated clones, along with the 10 week Fe2O3-exposed cells, maintained their transformed phenotypes, even after repeated (12-30) passages and freezing. Further studies using fluorescent imaging and protein expression analysis suggested that potential disruption to iron homeostasis gave rise to increased intracellular iron and increased ROS production which is well-known to cause oxidative damage and promote cell transformation. Sub-chronic *in vitro* exposures with cancer hallmark screening using human primary cell models holds promise as a Tier I screening model for ENM-associated tumorigenesis.

2784 Vacuole Formation and Caspase-Dependent Apoptosis in Human Monocytes Caused by Exposure to Titanate Nanosheet

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Toxicities of various kinds of nano-sized materials have been studied recently, which have demonstrated inflammatory response and tumor development caused by exposure to those. Titanate nanosheet (TNS) is a representative sheet-like nano-sized material with depth of about 1 nano meter, and has been developed as a material for UV- or corrosive-resistant films, dielectric thin films and catalysts. Therefore, we examined effects of exposure to TNS on human peripheral blood mononuclear cells (PBMCs) or isolated CD14+ monocytes. TNS was synthesized from Ti(O-i-Pr)4 and NEt4OH by a method of liquid-phase synthesis. PBMCs were cultured with TNS and assayed for apoptosis by flow cytometry and observed for morphological changes. PBMCs showed annexin V+ PI- apoptotic cells after 7 days of culture with TNS at more than 2 µg/ml, in contrast to early apoptosis after 2 days of culture with asbestos. The addition of Q-VD-OPh, pan-caspase inhibitor suppressed apoptosis caused by exposure to TNS. TNS-exposed PBMCs showed vacuole-forming cells, which were positive for CD14. Isolated CD14+ monocytes generated vacuoles until after 1 day of the culture with TNS, and vacuoles enlarged by the day. The observation by transmission electron microscopy showed the existence of nano-sized materials with TNS-like shape inside vacuoles. TNS exposure affected endosome structures, visualized by pre-incubation with fluorescence-labeled dextran, and vacuoles in those monocytes included fluorescence dextran. TNS-exposed monocytes were sliced and analyzed by scanning electron microscopy and energy dispersion type X-ray spectroscopy. The inner surface of vacuoles involved some agglomerated body, which showed the presence of titanium. These results indicate a unique toxicity of TNS, in which TNS entered into endocytic pathway leading to the formation of vacuoles, followed by caspase-dependent apoptosis.

2785 Toxicological Evaluation of Cobalt Oxide Nanoparticles on HepG2

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Cobalt oxide (Co3O4) nanoparticles in nanomedicine and nanotechnology especially is due to their enhanced magnetic properties, even though Co has been reported to have high toxicity and could potentially be classified as a carcinogen by the International Agency for Research on Cancer (IARC). Indeed, Co based nanoparticles reportedly produce inflammation, apoptosis, and DNA damage. Therefore, we investigated the cytotoxicity, genotoxicity, oxidative damage, and apoptosis-induction of Co3O4 nanoparticles (40 nm) on the liver (HepG2 hepatocarcinoma cell). After 24 h exposure, the nanoparticles decreased cell viability at ≤100 µg/mL, while increasing viability at the concentrations more than 100 µg/mL. The nanoparticles induced DNA (≤1.5-fold of the negative control) and oxidative damage with increased malondialdehyde (MDA) and 8-hydroxydeoxyguanosine (8-OHdG) levels and decreased glutathione (GSH) levels. The nanoparticles also slightly induced apoptosis/necrosis (≤ 6.3 fold of the negative control). Their adverse effects should raise concern about their safety associated with their applications in consumer products. However, the obtained results need to be supported with *in vivo* studies to fully understand the mechanism of Co3O4 nanoparticle toxicity.

2786 Surface Reactivity and Cell-Particle Bio-Interactions Explain the Precocious Cytotoxicity of Copper Oxide Nanoparticles

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Copper oxide nanoparticles (nCuO) are promising substitutes of different toxic substances currently used for antimicrobial purposes but is already proven that they are also strong cytotoxic agents for mammalian cells. The aim of this work was to understand how differently synthetised nCuO could induce precocious cytotoxic effects depending on their chemical-physical properties. In particular, we focused the attention on cell-particle interaction and protein modulation in order to define potential markers of precocious effects by exposing A549 cells to a commercial form of nCuO in comparison to sonochemically prepared-one. Particles were fully characterised by HRTEM, XRD, TCA and

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Preface

This issue is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 55th Annual Meeting of the Society of Toxicology, held at the New Orleans Ernest N. Morial Convention Center, March 13–17, 2016.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 603.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 629.

The abstracts are reproduced as accepted by the Scientific Program Committee of the Society of Toxicology and appear in numerical sequence. Author names which are underlined in the author block indicate the author is a member of the Society of Toxicology. For example, J. Smith.

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