results showed that micronucleus induction by AgNPs was similar when evaluated using flow cytometry or microscope,whereas the induction by TiO_2NPs was different using the two methods due to TiO_2's fluorescence interference with the cytometry equipment. Cells with the mutated p53 gene were more sensitive to micronucleus induction by AgNPs than the p53 wild-type cells. The presence of serum during treatment increased the toxicity of AgNPs. The coatings of nanoparticles played an important role in the genotoxicity of AgNPs. These collective data highlight the importance of considering the unique properties of nanoparticles in assessing their genotoxicity using the *in vitro* micronucleus assay.

PS 1529 Optimization of a Three-Dimensional Airway Epithelium Co-Culture System As a Platform for Adverse Outcome Pathway Assessment of Engineered Nanomaterials

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Engineered nanomaterials (ENM), including silver nanoparticles (AgNP), are one of the largest groups of emerging toxicants. AgNP are used in consumer and medical products due to their antimicrobial properties, and have a potential to aerosolize through the manufacturing and use of these products. Toxicological studies have demonstrated that AgNP produce respiratory toxicity, which has been characterized by responses such as, activation, degranulation, inflammation, oxidative stress, and cytotoxicity. This study is developing three-dimensional airway epithelium co-culture systems (3D-AECS) as a platform to predict pathways of response to AgNP, and is using Adverse Outcome Pathways (AOP) to understand how these are conferred across multiple levels of biological organization–on the molecular, cellular, and organ system levels. 3D-AECS are being developed through a "tiered approach" that co-cultures murine tracheal epithelial cells with alveolar macrophages (MTEC/AM) under multiple differentiation states at the air-liquid interface (ALI) to determine the optimal system for use in AOP. In this preliminary study, we investigated 3D-AECS response to AgNP differentiated under normal and IL-13 conditions to reflect "healthy" and "diseased" phenotypes, respectively. To assess 3D-AECS response to AgNP, we quantified differences in: cytotoxicity, chemokine release, and gene expression related to cellular adaptive responses such as, organ/tissue injury, differentiation, and oxidative/endoplasmic reticulum (ER) stress. Dose-response relationships for cytotoxicity were observed across 3D-AECS differentiated under normal and IL-13 conditions. In normal differentiated 3D-AECS, cytotoxicity increased from 5.0% to 15.5% and 3.7% to 28.1% in A/J and C57BL/6J mice, respectively. In IL-13 differentiated 3D-AECS, cytotoxicity increased from 4.2% to 23.4% and 5.2% to 26.9% in A/J and C57BL/6J mice, respectively. This will influence the optimization of 3D-AECS to provide information on potency and mode of action for critical and relevant end points of respiratory toxicity.

PS 1530 Effects of Titanium Dioxide and Nickel Oxide Nanoparticles on Model Membrane Systems Determined by Time-Resolved Fluorescence Specroscopy

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The proliferation of nanotechnology is resulting in increased use of engineered nanomaterials (ENM). This rapid expansion of nanotechnology poses an increased risk of human exposure to ENM. Two ENM whose use has increased are titanium dioxide nanospheres (TiO_2-NS) and nickel oxide nanospheres (NiO-NS). While TiO_2-NS have been shown to be non-toxic, NiO-NS have been shown to have some toxicity and can produce an allergic reaction. Both of these materials are taken-up by phagocytic cells where they can accumulate. This provides an opportunity for these particles to interact with cellular lipid membranes. Fluorescence lifetime and time-resolved anisotropy, using appropriate lipid probes, can measure changes in lipid structural characteristics, such as lipid order and disorder. To investigate the interaction between TiO_2-NS and NiO-NS with lipid membranes, we used liposomes (100 nm diameter) synthesized with phospholipids with different head group charges. Liposomes consisted of either 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC) with a neutral head-group or 1,2-Dioleoyl-sn-glycero-3-phosphoserine (DOPS) with a negatively charged head-group. The solvatochromic fluorescence membrane probe Di-4-ANEPPDHQ was used to determine changes in lipid structure of the DOPC and DOPS liposomes. Fluorescence lifetime and time-resolved anisotropy were measured using a FLASC-1000 (Quantum Northwest) time-resolved fluorimeter after excitation with a 470-nm ps-pulsed laser. Initial fluorescence lifetime and time-resolved anisotropy of liposomes with Di-4-ANEPPDHQ were recorded. Then these liposomes were exposed to 50 µg/ml of either TiO-NS or NiO-NS. Anisotropy was measured every 30 minutes for 2 hours and fluorescence lifetime was measured after two hours. After 2 hours of incubation, the fluorescence lifetime was observed to decrease after NiO-NS exposure by 16.5% for DOPC and 13.5% for DOPS; TiO-NS had essentially no effect on either lipid. After 1 hour, the anisotropy was found to decrease in the DOPS liposomes by 9.6% with TiO-NS exposure and 10.3% with NiO-NS exposure, with little further change after 2 hours. DOPC liposomes exhibited no change in anisotropy after 2 hours. Taken together, these results support the notion that NP have defined impacts on membrane properties that may account for initial mechanisms of toxicity. *(Funding: NIH R01ES023209, 1F32ES027324, P30GM103338 and P20GM103546)*

PS 1531 Genotoxicity from As-Produced and Post-Production Modified Multi-Walled Carbon Nanotubes

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Pristine, as-produced multi-walled carbon nanotubes (AP-MW) are modified with coatings such as polymers (PC-MW) or aluminum oxide (AL-MW) for varied commercial applications. In order to determine the potential effect of coating on genotoxicity, we measured the genotoxic response of AP-MW from three companies (AP-MW1, 2 and 3) and their coated counterparts (PC-MW1 & 2 and AL-MW3). Genotoxicity was measured in immortalized (BEAS-2B) and primary lung epithelial cells (SAEC) either 24 or 72 hours after exposure using micronuclei assay with pancentromere staining, mitotic spindle analysis, and cell cycle analysis. There was significant necrosis at the highest dose of 24 µg/mL with the exception of AP-MW1. All MWs produced significantly greater amounts of micronuclei after exposure to 2.4 µg/mL in both cell types, however, there was a significant difference between AP-MW2 and PC-MW2 in the BEAS-2B cell. Genetic damage was due to both clastogenic and aneugenic effects in both cell types. Analysis of the mitotic spindle demonstrated a dose-dependent increase of mitotic spindle aberrations that were predominately monopolar. No significant apoptosis was observed in the BEAS-2B cell type. SAEC cells exposed to AL-MW3 showed significant apoptosis. Exposure to 2.4 µg/mL of each material produced significant arrests in the G1 and G2 phases of the cell cycle. These data indicate that all tested materials produced a genotoxic response. The coating did not affect the response in comparison to their respective as-produced counterpart.

PS 1532 Mechanism-Based Genotoxicity Screening of Nanomaterials Using the Toxtracker Panel of Reporter Cell Lines

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The rapid development of the engineered nanomaterial (ENM) manufacturing industry has accelerated the incorporation of ENMs into a wide variety of consumer products across the globe. It is therefore essential to have rapid and robust analytical metrology in place that can be used to critically assess and/or predict the cytotoxicity, as well as the potential genotoxicity of these ENMs. Recently, the ToxTracker reporter assay was identified as one of the emerging methods for genetic toxicology assessment of ENMs (Nelson et al, Mutagenesis 2016). We have evaluated the ability of ToxTracker to identify the hazardous properties and underlying mechanisms of a panel of 24 metal and non-metallic ENMs. First we evaluated the ability of the ToxTracker reporter cell lines to identify the hazardous properties of a panel of metal oxide- and Ag nanoparticles (NPs), as well as a selection of non-metallic materials (diesel, carbon nanotubes and quartz). The reporter cells were able to take up NPs, and furthermore, exposure to CuO, NiO and ZnO NPs as well as to quartz (used as a benchmark particle) resulted in activation of the Srxn1-GFP oxidative stress reporter, although only at high cytotoxicity for ZnO. Next, we extended the toxicity screening of ENMs by investigating CdTe quantum dots (QD) of various sizes (between 1.5 and 9 nm) and found clear size-dependent effects in terms of cytotoxicity and oxidative stress reporter activation and cell viability. The lowest LC50 values were observed with the smallest QDs (1.5nm). For the ENMs tested in this study, oxidative stress appeared to be the primary mechanism of (geno)toxicity. Interestingly, cobalt (Co) NPs activated the Rtkn-GFP reporter that is associated with DNA strand breaks, thereby suggesting induction of DNA damage. Cobalt dust has been reported to cause lung cancer in 2-year inhalation studies in rats and mice. None of the tested ENMs induced the Bscl2-GFP DNA damage reporter indicating that none of the tested ENMs could directly bind to DNA or inter-


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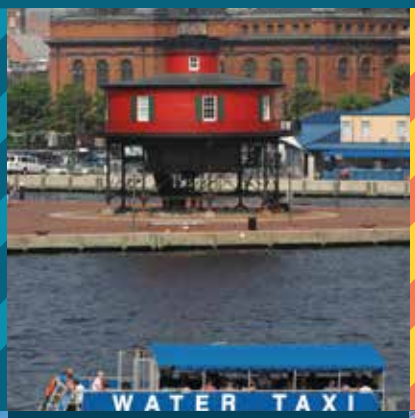

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