of 12 types (40, 60, 80 and 100 nm coated with citrate, polyvinylpyrrolidone (PVP), and branched polyethyleneimine (BPEI)) of AgNPs on organelle morphology in human retinal pigmented epithelial (ARPE-19) cells. AgNPs (0.1-30 µg/ml) were applied to the cells seeded in a 384-well format, with silver nitrate acting as silver ionization control. The Distorted Grid (DG) model was used to estimate the cellular delivered dose. After 24 hrs of treatment, cells were live-labeled with MitoTracker (mitochondria), fixed, permeabilized and labeled with Hoechst-33342 (nuclei), SYTO14 (nucleoli) and fluorescent conjugates of concanavalin A (ER), phalloidin (actin cytoskeleton), and wheat germ agglutinin (Golgi/plasma membrane). A multiplexed cell viability and apoptosis assay was run in parallel. Cells were imaged using an Opera Phenix High Content Screening System and profiled using Harmony High Content Analysis software. Approximately 1200 morphological features were measured per cell and summarized to the well level for analysis. The DG model predicted that the fraction of AgNP deposited on cells increased with particle size and differed based on coating (BPEI > citrate > PVP). Phenotypic profiling showed that all AgNP types affected the organelle morphology in a concentration-dependent manner, with over 600 features having benchmark doses below the threshold for cytotoxicity. The pattern of changes in cell morphology differed across coating agents and silver nitrate. Citrate coated AgNPs showed the most pronounced effects below the cytotoxic threshold. 60 nm PVP had fewer effects on cell morphology than other coatings, yet apoptosis was observed at an estimated delivered dose as low as 1.45 µg/ml. This screening method may inform subsequent assay selection by highlighting the intracellular regions affected by AgNPs of interest. *This abstract does not necessarily reflect US EPA policy.*

2200 Amorphous Silica Coating Protects against Iron Oxide Nanoparticle-Induced Cell Transformation and Genotoxicity

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Iron oxide nanoparticles (IONP) have a wide range of uses in biotechnology, medicine, and transportation. However, very little is known about their potential adverse health effects following human exposure. Some evidence suggests that dissolution of IONP following endocytosis into cells may disrupt iron homeostasis, resulting in genotoxicity and neoplastic-like cellular transformation. Surface modification of IONP, such as an amorphous silica coating, may impact subsequent adverse outcomes by reducing particle dissolution. The main objective of this study was to assess IONP low dose, long term exposure effects, including carcinogenic potential, as well as the utility of an amorphous silica coating in reducing or preventing these outcomes. Human bronchial epithelial cells (Beas2B) were continuously exposed to nFe_2O_3 or nano-SiO_2 coated nFe_2O_3 (SiO_2-nFe_2O_3) for up to 6.5 months at an occupationally relevant low dose (0.6 µg/cm^2 or 2.88 µg/mL) and evaluated over time for indications of neoplastic-like transformation and its underlying mechanism. Transformation was compared to that induced by gas metal arc mild steel welding fumes (GMA-WF), which were recently re-classified as a Group 1 total human carcinogen, and are composed of roughly 80% iron/iron oxide. Our results showed that beginning at four months, nFe_2O_3-exposed Beas2B underwent neoplastic-like transformation, as indicated by increased cell proliferation and attachment-independent colony formation. These outcomes correlated with nFe_2O_3 dissolution, increased intracellular iron, and genotoxicity, as well as significant changes in pathways related to DNA damage repair and autophagic processes. nFe_2O_3-induced transformation also closely matched that GMA-WF induced transformation SiO_2-nFe_2O_3 treatment, however, did not induce any changes in the above parameters. Overall, our results indicated potential carcinogenic risk of nFe_2O_3 associated with particle dissolution, iron homeostasis disruption, and changes in autophagy and DNA damage repair pathways, which were reduced with an amorphous silica surface coating. This study shows the potential utility of a "safe by design" hazard reduction strategy, to alter particle physicochemical properties based on mode of toxicity to reduce risk.

2201 Potential Role of Thioredoxin-Interacting Protein in Silver Nanoparticle-Mediated Mast Cell Degranulation

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Silver nanoparticles (AgNPs) are being incorporated into a variety of consumer and medical products primarily due to their antimicrobial properties. This will lead to a significant rise in exposure to the general population; however, our understanding of the potential adverse health effects of AgNPs is still minimal. We have previously demonstrated that AgNPs induce a non-IgE mediated degranulation of mast cells. Importantly, we have also shown a strong genetic contribution to AgNP driven mast cell degranulation. RNA sequencing performed on bone marrow-derived mast cells (BMMCs) from high-responding (C57BL/6) and low-responding (LP/J) strains of mice demonstrated a significant increase in thioredoxin-interacting protein (*txnip*) in the low-responding strain after exposure to 20nm AgNP. We therefore explored the role of TXNIP in mast cells to determine its potential regulatory role in AgNP-mediated degranulation. At one hour following exposure to AgNP, *txnip* mRNA levels were increased in LP/J but not C57BL/6 which confirms RNA sequencing results. Protein levels of TXNIP were similarly increased in LP/J BMMCs at six hours post-exposure while protein levels were significantly decreased in C57BL/6 BMMCs. siRNA knockdown of TXNIP resulted in a trend towards increased mast cell degranulation. Using a Seahorse XF analyzer, we found that BMMCs from low- and high-responding strains possess varying glycolytic capacities in response to AgNP exposure possibly implicating TXNIP as a regulatory protein for cellular metabolism during mast cell degranulation. Our data suggests that TXNIP plays a role in non-IgE mediated mast cell degranulation initiated by exposure to 20nm AgNP and may possibly modulate cellular metabolism.

2202 *In Vitro* Dermal Toxicity of Redox-Active Metal Nanocatalysts

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Nanocatalysts (NCT) represent the convergence of catalysts, a mature technology with a new one, nanotechnology. NCT is a rapidly growing field that involves the use of nanomaterials as catalysts for a variety of catalytic applications. Since metal nanoparticles (MeNP) have a large surface-to-volume ratio compared to bulk materials, they are attractive candidates for use as catalysts. A number of redox-active MeNP and their oxides (MeO) including nickel (Ni) and cobalt (Co) are widely used. The physical nature and reactive surface properties of some of these may affect their ability to induce dermal toxicity thus causing adverse skin reactions. We hypothesize that toxicity of Me/MeO NP occurs via their ability to initiate oxidative stress, thereby inducing redox-sensitive transcription factors and triggering inflammation. Moreover, due to the skin's susceptibility to UV radiation, it is important to evaluate whether Me/MeO NP enhance the adverse effects of UVB. To test these hypotheses, the effects of Ni, Co, NiO, Co_3O_4 and CoO alone (0-26 µg/cm^2) and co-exposed with UVB (4KJ/m^2) were studied *in vitro* and *in situ* using murine epidermal cells (JB6 P+/+) and an engineered human skin construct (EpiDerm FT). Cell exposure to Me/MeO NP resulted in a dose- and time-dependent loss of cell viability, cell damage, oxidative stress and activation of AP-1/NF-κB. Co-exposure of Me/MeO NP with UVB ensued in amplification of the observed effects. Exposure of EpiDerm FT to Me/MeO NP caused tissue damage, oxidative stress and accumulation of inflammatory mediators. Hierarchical cluster analysis resulted in two major clusters separating cytokines production related to inflammatory cell recruitment (more intense) and T_H2-type/regulatory immune responses (dimmed). UVB exposure alone induced significant tissue damage and secretion of cytokines/chemokines. Ni compounds drastically enhanced the post-UV treatment LDH release and secretion across the whole cytokine spectrum, while Co oxides prompted much weaker reaction. Interestingly, inflammatory cytokine/chemokine levels upon exposure to Me/MeO NPs, with or without UVB pre-treatment, followed similar trends compared to cell/tissue damage i.e., NiO>Ni>Co_3O_4>CoO and correlated with their size. Altogether, these results clearly indicate that some of the Me/MeO NP could induce cytotoxicity, oxidative stress and inflammation, and may potentially enhance response caused by UVB pre-treatment. *Disclaimer: The findings and conclusions of this report are those of the authors and do not necessarily reflect those of National Institute for Occupational Safety and Health.*

2203 Comparative *In Vitro* Study of Adverse Pro-neoplastic Potential of Tremolite Asbestos and Its Cleavage Fragments in Human Epithelial (BEAS-2B) and Mesothelial (MET-5A) Cells

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The fibrous form of tremolite is one of the six recognized types of asbestos. It is known that inhalation of respirable tremolite fibers (TF) can cause asbestosis, lung cancer and both pleural and peritoneal mesothelioma. Tremolite also occurs in a non-fibrous habitat that can be mechanically broken into cleavage fragments (CF) which can meet the criteria for fibers. Despite the considerable amount of work showing that CF are less potent in their damaging effects than asbestos fibers, little data exists on the adverse effects of well-characterized tremolite CF. The present study was designed to reveal the potential pro-carcinogenic manifestations of respirable TF and correspond-

ing CF (with similar median length but different width/aspect ratio) using *in vitro* models. Sub-chronic exposures of human epithelial (BEAS-2B) and mesothelial (MET-5A) cells - both target cells of the respective lung cancer and malignant mesothelioma - to TF and CF were employed in the current study. Cells were evaluated for the presence of several cancer hallmarks indicating the neoplastic transformation following continuous exposure to the sub-toxic concentration of TF and CF for 5 weeks. TF-exposed cells, both BEAS-2B and MET-5A, revealed a neoplastic-like transformation phenotype characterized by significant increase in proliferation, morphological transformation, invasion/migration and anchorage-independent growth compared to controls. In contrast, no anchorage-independent growth was observed in CF-exposed cells although an increase in proliferation, morphological transformation and migration/invasion was detected albeit at lower intensity compared to TF. Additionally, CF had no impact on apoptosis susceptibility while TF caused increased apoptosis in MET-5A cells and its inhibition in BEAS-2B cells. Both TF and CF induced oxidative DNA damage albeit with a stronger effect in TF-exposed cells. Analysis of inflammatory responses using a cytokines/chemokines clustering approach suggested cell-type specific effects to TF and CF exposures as well as treatment related differences. Overall, our data are compatible with the interpretation that tremolite asbestos fibers demonstrated higher neoplastic transformation potential compared to CF (at the same mass dose) in both epithelial and mesothelial cells. *Disclaimer: The findings and conclusions of this report are those of the authors and do not necessarily reflect those of National Institute for Occupational Safety and Health.*

2204 Effects of Multiwalled Carbon Nanotube Accumulation on Macrophage Cell Viability and Proliferation *In Vitro*

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The range of applications for multi-walled carbon nanotubes (MWNTs), from electronics to medicine, is increasing their global production despite an incomplete understanding of their toxicological potential. A recent study reported that more than 99% of all nanoparticles administered *in vivo* are sequestered by macrophages, but the types and severity of effects induced by MWNT accumulation in macrophage cells is not well understood. Our previous work showed that macrophages preferentially accumulate ~100X more Pluronic® F108-coated carboxylated MWNTs (cMWNTs) than nonfunctionalized pristine MWNTs (pMWNTs) (Wang et. al., Nanotoxicology, 2018, DOI: 10.1080/17435390.2018.1472309). Furthermore, cMWNT accumulation in RAW 264.7 cells after a 20h exposure impaired subsequent phagocytic function. Our recent work focuses on the effects of cMWNTs on other macrophage cell functions, specifically cell proliferation and viability. To assess cell proliferation, RAW 264.7 cells were treated with varying concentrations of cMWNTs or pMWNTs and incubated at 37°C for 24, 48, or 72 hours. The cells were then washed to remove MWNTs in the media and proliferation was measured with a crystal violet assay. The results showed that neither cMWNT nor pMWNT accumulation significantly affected cell proliferation after a 24h exposure, but cell proliferation was reduced by as much as 88% and 95% after exposure to cMWNTs at 200 µg/mL for 48h and 72h, respectively. The severity by which cell proliferation decreased between 24h and 72h of exposure raised the question of whether 24h exposure to cMWNTs affected cell viability in ways undetected by the cell proliferation assay. Consequently, colony formation assays were conducted in which RAW 264.7 cells were treated either with pMWNTs at 100 or 200µg/mL, or with cMWNTs at concentrations ranging from 25 to 200µg/mL and incubated at 37°C for 24h. The cells were then washed, harvested, seeded at a density of 1000 cells per plate, and incubated at 37°C in media free of MWNTs for 10 days, after which the colonies were stained and counted. There were 371 ± 22, 248 ± 27, and 187 ± 13 colonies per plate for the control, pMWNT-treated, and cMWNT-treated cells, respectively, which suggested that cMWNT accumulation over 24h impaired cell viability by 49.6%. Additional experiments will explore the effects of cMWNTs on reactive oxygen species and apoptosis.

2205 Evaluation of Cytotoxicity Potential of 6-Thioguanine Loaded Chitosan Nanoparticles with or without Curcumin

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CCancer is the second leading cause of mortality in the world. Cancer nanotherapeutics are rapidly progressing and being implemented to overcome several limitations of conventional drug delivery systems. The objective of the study was to synthesize 6-thioguanine (6-TG) loaded chitosan nanoparticles (CNPs) and to evaluate the cytotoxicity with or without curcumin (CUR) on two cancer cell lines *viz.* Breast adenocarcinoma (MCF-7) and Ovarian teratocarcinoma (PA-1). 6-TG loaded CNPs were formulated by ionic-gelation method. Morphologically the 6-TG loaded CNPs were spherical in shape and showed mean size, PDI, zeta potential and Entrapment efficiency of 261.63 ± 6.01 nm, 0.35 ± 0.10, 15.97 ± 0.46 mV and 44.27% respectively. MTT [3-(4, 5- dimethylthiazolyl- 2)-2, 5- diphenyltetrazolium bromide] assay was conducted on MCF-7 and PA-1 cell lines at 48 h incubation for cytotoxic evaluation. IC_{50} values of 6-TG, 6-TG loaded CNPs and CUR for MCF-7 were 23.09 µM, 17.82 µM and 15.73 µM respectively. Likewise, IC_{50} values of 6-TG, 6-TG loaded CNPs and CUR for PA-1 were 5.81 µM, 3.92 µM and 12.89 µM respectively. Combination of 6-TG loaded CNPs (IC_{25}) with CUR (IC_{25}) on PA-1 and MCF-7 showed percent cell viability of 43.67 ± 0.02 and 49.77 ± 0.05 respectively. This study suggests that the cytotoxicity of 6-TG and 6-TG loaded CNPs is dose-dependent and 6-TG loaded CNPs proved to be more effective (~1.5-fold high anticancer efficacy) than that of 6-TG against PA-1 cells. Further, combination of 6-TG loaded CNPs with CUR showed synergistic cytotoxicity *i.e.* enhanced anticancer efficacy on PA-1 cancer cells.

2206 Bioactivity of Multiwalled Carbon Nanotube Mixtures with Multiple Aspect Ratios

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Multi-walled carbon nanotube (MWCNT) composites have vastly superior mechanical and structural properties compared to conventional materials. In order to be cost effective and to improve the distribution in the composite matrix, MWCNTs produced in large volume with multi-aspect ratios are utilized. Past research identified pulmonary health effects and the molecular mechanisms associated with exposure to mostly uniformly dimensional tubes; however, much is unknown concerning exposure associated with mixtures containing multi-aspect ratio tubes. In order to investigate this concern, we evaluated the toxicity profile of two multi-aspect-ratio MWCNT mixtures (MWCNT-1 and MWCNT-2) and compared them with the toxicity profile of more uniform and well-characterized MWCNT (Mitsui-7) and carbon black (CB, Printex-90) samples. Automated field emission scanning electron microscopic analysis showed that the MWCNT mixtures had a wide distribution of lengths from a few nanometers up to 20 µm and diameters that change according to length. The Mitsui-7 were more uniform with a diameter ~50 nm and the CB had a diameter of ~20 nm. Cytotoxicity and cell proliferation was assessed in human monocytic cells (THP-1) at 0 - 120 µg/ml and in primary human fibroblast cells (PHF) at 0 - 16 µg/ml. NFκB (nuclear factor kappa-light-chain-enhancer of activated B cells)-induced inflammatory potential was screened using THP-1 reporter cells. THP-1 WT and NLRP3-deficient cells were used to screen for inflammasome activation. Acellular oxidative stress potential of the material correlated with the fold increase in *in vitro* NFκB activation and oxidative stress induction, measured using a dichlorofluorescin-diacetate assay. The extracellular remodeling and fibroblast transformation potential was evaluated by measuring collagen 1, α-smooth muscle actin, and TGF-B in PHF cells. Given the toxicity and the metrics for molecular initiating events, the MWCNT-2 mixture was the most bioactive material followed by Mitsui-7 > CB > MWCNT-1. We conclude that it was not possible to fit all multi-aspect-ratio MWCNTs into a universal toxicity profile. Ongoing extensive physiochemical characterization could elucidate the key confounders influencing the toxicity profiles of such a multi-aspect-ratio MWCNT mixture.

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