PS 603a Assessment of the *In Vitro* Irritant Contact Dermatitis Potential of Metal-Containing Nanoparticles Using Human-Derived Epidermal Keratinocytes

M. F. Hughes[1] and V. Miyani[2]. [1]*ORD/NHEERL, US EPA, Research Triangle Park, NC and* [2]*Student Services Contractor, Morrisville, NC.*

Dermal exposure to metals may result in irritant contact dermatitis. The objective of this study was to examine the in vitro potential of metal-containing nanoparticles to elicit irritant dermatitis in human-derived epidermal keratinocytes (HDEK). These cells are cultured and form a multi-layered, highly differentiated model of human skin. Ag (14), TiO_2 (7) and CeO_2 (3) nanoparticles of various sizes (10 nm – 214 nm) were used in this study. The Ag particles were capped with either citrate or polyvinylpyrrolidone/citrate (PVP) or coated with silica. The Ag nanoparticles were suspended in saline and the TiO_2 and CeO_2 particles were suspended in cell culture media containing 10% fetal bovine serum. Stock suspensions of these particles were dispersed using a probe sonicator. The particles (1 mg/ml, 30 µl) were transferred to the epidermal surface of the HDEK in 24-well plates. Three wells of HDEK were tested per particle. The positive control was 5% sodium dodecyl sulfate (SDS) and the negative control was either saline or culture media. After 1 hr exposure at 37 °C the HDEK were washed with saline. The HDEK were then incubated for 42 hr at 37 °C with an exchange of media at 24 hr. Viability of the HDEK was assessed using the MTT assay. A test substance is considered an irritant in this assay if the HDEK viability is less than 50%. The mean viability for the SDS treated HDEK was 7.8%. The lowest viabilities (mean ± SD) for the nanoparticle treated HDEK were: Ag 75 nm PVP, 91.9 ± 3.8%; Ag 50 nm silica coated, 91.9 ± 3.6%; Ag 80 nm silica coated, 91.9 ± 5.3%; TiO_2 22 nm (anatase), 93.2 ± 22.2%; CeO_2 58 nm, 91.3 ± 3.9%. Under the in vitro conditions used in this study, the Ag, Ti and Ce nanoparticles examined were not dermal irritants to the HDEK. The formed stratum corneum of the HDEK may limit penetration of these nanoparticles to induce inflammation and cell death or their inherent dermal irritancy potential is very low. (This abstract does not necessarily represent U.S. EPA policy.)

PS 603b A Simple Approach for the Determination of Nanoparticle-Induced Cytotoxicity *In Vitro*

Q. Gu[1], S. Lantz-McPeak[1], E. Cuevas[1], H. Rosas-Hernandez[1], S. F. Ali[1], S. Sarkar[1], M. G. Paule[1] and Y. Zhang[2]. [1]*Division of Neurotoxicology, FDA/NCTR, Jefferson, AR and* [2]*Nanotechnology Core Facility, FDA/NCTR, Jefferson, AR.*

Emerging new materials utilizing nanoparticles (NPs) are being increasingly incorporated into consumer products. Thus, elucidating the potential toxicities associated with NP-exposure will lead to a better evaluation of the undesired biological impacts of NP-based materials. Here we report a simple approach for the assessment of NP-induced cytotoxicity *in vitro* using Fluoro-Jade C (FJ-C), which has been extensively utilized in *in vivo* neurotoxicity studies where they have been shown to label dead and dying neurons. In the present study, different types of cell cultures including rat brain micro-vessel endothelial cells, bovine brain micro-vessel endothelial cells, and the human cell line SH-SY5Y were examined, and potential cytotoxic effects of silver-nanoparticles (Ag-NPs) and gold-nanoparticles (Au-NPs) were tested. Whereas incubation with 20 nm diameter citrate-Ag-NPs for 24 hours induced a dose-dependent increase in the number of FJ-C labeled cells, incubation with 7 nm diameter tannate-Au-NPs for 24 hours showed few changes even at concentrations higher than those used for Ag-NP studies. The dose-dependent i*in vitro* cytotoxicity of Ag-NPs and the lack thereof for Au-NPs were further confirmed by two commonly used cytotoxicity assays, the lactate dehydrogenase (LDH) and 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) assays. In addition to the examination of Ag-NP-induced cytotoxicity, two well known toxicants, cadmium and Thimerosal, were also employed to induce cell death *in vitro* and both elicited a significant increase in the number of FJ-C labeled cells. These results suggest that the FJ-C-based approach can provide an easily obtained assessment of NP-induced cytotoxicity and has the potential to lead to high-throughput *in vitro* screening and analyses. This simple FJ-C labeling approach may have broad application for the assessment of *in vitro* cytotoxicity following NP-exposure.

Supported by FDA/NCTR Protocols E07460 and E7524.

PS 603c Influence of Nanoparticles on Cell Death and Cell Cycle in A549 Cells

Y. Huang[1], L. M. Tolliver[1], F. S. Hou[2], R. S. Aronstam[1] and H. Lee[3]. [1]*Biological Sciences, Missouri U. of Science and Technology, Rolla, MO,* [2]*Department of Clinical Laboratory Science, Marquette University, Milwaukee, WI and* [3]*Department of Natural Resources and Environmental Studies, National Dong Hwa University, Hualien, Taiwan.*

Although frequently used in industrial and biomedical applications, fourth period metal oxide nanoparticles (NPs) display varying degrees of cytotoxicity, of which the mechanisms need further exploration. In the past, we established a trend of cytotoxicity of transition metal oxide NPs. Three distinct "tiers" of cytotoxicity was observed: low (TiO2, Cr2O3 and Fe2O3), moderate (Mn2O3 and NiO) and high (CuO and ZnO). In this report, we investigated influence of these seven NPs on cellular ROS generation, cell death and cell cycle in A549 cells. ROS generation was measured by DCFH-DA. TiO2, Cr2O3 and Fe2O3 did not elevate ROS while Mn2O3 and NiO, CuO and ZnO increased ROS by several folds. The apoptotic dyes Annexin-V conjugated fluoroisothiocyanate (FITC) and 7-Aminoactinomycin D (7-AAD) were used to characterize cell death of NP-treated cells through immunofluorescence microscopy and quantify it through flow cytometry. The flow cytometry results mimicked the same general trend of low, moderate and high toxicity. The combined populations of early apoptotic cells and late apoptotic/early necrotic cells treated with the highest concentrations of CuO (20 µg/mL) and ZnO (25 µg/mL) were 71.8%±7.6% and 59.8%±4.9%, respectively. The combined populations of early apoptotic cells and late apoptotic/early necrotic cells of the rest of five NPs range from 3.2±0.85%. The degree of apoptosis and necrosis corresponded with severity of cytotoxicity. Different NPs differentially affect different phases of cell cycle. One notable observation was that zeta potentials of NPs varied significantly in water (-7 to -34 mV), PBS solution (-22 to -30 mV), and cell medium (-6.4 to -9.2 mV). The potentials do not correlate with the trend of cytotoxicity.

PS 603d Molecular Responses to MWCNT Pulmonary Exposure at Relevant Workplace Exposure Levels

P. C. Zeidler-Erdely[1], S. Shah[2], S. Tugendreich[2], S. Bilgesu[1], T. Hulderman[1], J. Cumpston[1], D. G. Frazer[1], W. McKinney[1], B. T. Chen[1], V. Castranova[1] and A. Erdely[1]. [1]*HELD, NIOSH, Morgantown, WV and* [2]*Ingenuity Systems, Inc., Redwood City, CA.*

Pulmonary toxicity from multi-walled carbon nanotube (MWCNT) inhalation exposure is well described and includes lung epithelial hyperplasia, inflammation, and fibrosis. However, at levels relevant to workplace exposures the contributing molecular mechanisms are not well described. To address this question, a 19d inhalation exposure to MWCNT in C57BL/6J mice with daily alveolar depositions of 2340 ng (=1000 d of human exposure), 234 ng (=100 d), and 23.4 ng (=10d) was conducted. Cumulatively, these doses represent 76, 7.6, and 0.76 yr for the high, middle, and low dose, respectively, for a worker exposed to an inhalable concentration of 10 µg/m3 (average exposure from worker sampling at 8 different MWCNT sites; MMAD 5.5 µm) for 8 h/d for 250 d/yr. Mice were sacrificed at 0, 28, and 84 d post-exposure. RNA was isolated from left lung lobes and global expression analysis was performed with subsequent Ingenuity Pathway Analysis (IPA). Results indicated marked inflammation at the high dose that was sustained through 84 d post-exposure. Markers associated with pathological changes, such as fibrotic growth factors (TGFb, EGF, PDGF), were also a feature of the high dose. The middle dose was also associated with markers of inflammation (e.g. IL-6, IL-1b) but to a lesser extent and not as sustained when compared to the high dose. The middle dose showed indications of increased fibrotic markers at 0 and 28 d by BioProfiler analysis, but the response was not sustained and was absent at 84 d. The low dose showed minimal expression changes that did not form any major networks of activation. These results confirm the potential of MWCNT to induce molecular mechanisms associated with a marked inflammatory and pathological response in exposed mice. Based on exposure predictions, considerable years of exposure may be necessary at facilities operating at an inhalable concentration of 10 µg/m3 or below to elicit marked molecular changes.

The Toxicologist

Supplement to *Toxicological Sciences*

53rd Annual Meeting and ToxExpo™

March 23–27, 2014 • Phoenix, Arizona

An Official Journal of the Society of Toxicology

SOT | Society of Toxicology

Creating a Safer and Healthier World by Advancing the Science of Toxicology

www.toxicology.org

OXFORD UNIVERSITY PRESS

ISSN 1096-6080
Volume 138, Issue 1
March 2014

www.toxsci.oxfordjournals.org