buffered formalin for histopathological analysis. Preliminary morphometric data indicate slight reduction in body and lung weight. Measure of total protein also decreased in rats that ingested TDNF. Anatomical assessment of the pulmone indicate collapsed lung, bronchiolar arteriole hyperplasia, mild bronchiolar and alveolar hemorrhage, perivascular edema. The study is still ongoing to investigate genome-wide expression of transcripts in the lung.

1522 Positively Charged Nanoparticles Lead to Artifacts in a *C. elegans* Toxicity Assay

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The unique properties of engineered nanoparticles (ENPs) may lead to unforeseen interactions and artifacts in toxicity assays that were originally designed for soluble chemicals. Yet, there is a lack of test methods specifically tailored to ENPs, which has hindered efforts to understand the potential environmental consequences of ENP release. Here, we detail our efforts to adapt a *C. elegans* toxicity assay for use with ENPs, including a cause-and-effect analysis to identify potential sources of error followed by a sensitivity analysis to quantify the error. We found that shaking plates during the assay decreased growth by as much as 36% and changing the concentration of feed in the assay greatly altered the toxicity of the positive control, benzylcetyldimethylammonium chloride (BAC-C16). We also found that three commonly used *C. elegans* media formulations produced similar results in the toxicity assay, allowing for flexibility to suit ENP characteristics and reduce agglomeration. Additionally, in our testing we discovered that positively charged polystyrene nanoparticles (PSNPs) elicit toxic effects on *C. elegans*. However, those impacts were due to an interaction with *E. coli* that is used as feed in the assay and impacts on growth were more variable for PSNPs (52%) compared to BAC-C16 (9%). *E. coli* and positively charged PSNPs formed heteroagglomerates, leading to large particles that the nematodes cannot ingest. This, in turn, reduces growth and reproduction. We repeated this test using Au ENPs with coatings ranging from positive to negative and found similar results with our positively charged Au ENPs compared to PSNPs. In contrast, analysis of nine other Au nanoparticles that were neutrally or negatively charged did not show particle heteroagglomeration nor toxicity to *C. elegans*. We were able to quantify the *E. coli* aggregation via turbidity measurements and present an alternative assay, which does not include bacteria, to accommodate positively charge ENPs.

1523 Sphingosine Kinase 1 Deficiency Protects Against Particle-induced Lysosome Membrane Permeabilization and NLRP3 Inflammasome Activity in Macrophages

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Lysosome membrane permeabilization precedes activation of the NLRP3 Inflammasome and release of associated inflammatory cytokines including IL-1β. Particle exposure is known to induce lysosome membrane permeabilization in macrophages through mechanisms that have not yet been elucidated. Sphingosine kinases have been implicated in lysosome destabilization during apoptosis, but their role following particle exposure is not known. In this study, the contribution of sphingosine kinase 1 to particle-induced lysosome membrane permeabilization was addressed in bone marrow derived macrophages from WT and sphingosine kinase 1 KO mice. Measuring cathepsin and N-acetyl-beta-D-glucosaminidase activity in the cytosolic fraction, following selective digitonin extraction, was used to assess lysosome membrane permeabilization after exposure to multiple types of particles including: titanium nanobelts, titanium nanospheres, nickel oxide nanospheres, multi-walled carbon nanotubes, crocidolite asbestos, and crystalline silica. Sphingosine kinase 1 KO macrophages had significantly less cytosolic cathepsin and N-acetyl-beta-D-glucosaminidase activity than WT with most particle exposures. IL-1β production and cytotoxicity (LDH) were also less in sphingosine kinase 1 KO macrophages 24 hours after particle exposure. Addition of the inhibitor SPHK I2, which targets all sphingosine kinases, further decreased cytokine production from sphingosine kinase 1 KO macrophages, suggesting partial compensation by sphingosine kinase 2. Together, these results show a partial role for sphingosine kinases in mechanisms responsible for particle-induced lysosome membrane permeabilization.

1524 Examining the Hepatic Effect of TiO2 Nano Fiber Ingestion in Sprague Dawley Rats

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A nanomaterial refers to a material having structures in the nanometer size range. These materials differ from bulk materials in the way in which they behave. That is they exhibit a different scaling property and quantum effects thus affecting the chemical reactivity of materials including their mechanical, optical, electric, and magnetic properties. As a consequence nanomaterials have applications in electronics, transportation and telecommunication, imaging, biomedical applications, pollution remediation, cosmetics, coatings, as insulation materials and as well as nanocomposites. Specifically, titanium dioxide nanomaterials has been widely employed in pigments, sunscrees, pains, ointments, toothpaste and photocatalytic splitting of water. The goal of the present study is to examine hepatic effects associated with the ingestion of TiO2 nanofiber (TDNF). TDNF was fabricated via electrospinning method, followed by dissolution in water through the agitation. Six to seven weeks old male Sprague Dawley rats ingested 0, 10, 15 ppm twice a week for a total of 0 ppm, 40, 60 ppm TDNF for the duration of the study. At the end of the treatment period, animals were euthanized via CO2 asphyxiation. Blood was drawn via cardiac puncture. The liver and other organs were autopsied. Some of the tissues were fixed in neutral buffered saline and the others frozen in liquid nitrogen until analysis. Clinical chemistry assessment of alanine transferase and total protein will be carried out. Histopathological analysis of the liver showed no significant difference between groups, though moderate glycogen accumulation were noted in the exposed animals. Serum total protein levels were dose-dependently reduced in treated groups. Additional study will involve proteomic assessment of bulk proteins will be performed to understand and identify health impact associated with exposure to TDNF.

1525 Stable Isotope Method to Measure Drug Release From Nanomedicines

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Existing methods to measure nanomedicine drug release in biological matrices are inadequate. A novel drug release method utilizing stable isotope dilution has been developed. Stable isotope-labeled drug is spiked into plasma containing nanomedicine. The labeled drug equilibrates with plasma components identical to the normoisotopic drug released from the nanomedicine formulation. Therefore, the ultrafilterable fraction of the isotope-labeled drug represents a reliable measure of free normoisotopic drug fraction in plasma, and can be used to calculate nanomedicine encapsulated and unencapsulated drug fractions. To demonstrate the utility of this method, we performed a plasma drug release study with both a fast releasing commercial docetaxel formulation, Taxotere®, and a delayed releasing nanomicellar formulation of a docetaxel prodrug, Procet 8. The instability of the unencapsulated prodrug in plasma allowed us to compare our calculated prodrug release and docetaxel conversion with the actual docetaxel concentration measured directly without fractionation. Drug release estimates for the fast releasing Taxotere formulation demonstrated accuracy deviation and precision (%CV) of <15%. For the controlled release Procet 8 formulation, we calculated a slow release and conversion of the prodrug in rat plasma that was highly correlated with the direct docetaxel measurement (R2=0.98). We believe this method will have tremendous utility in development and regulatory evaluation of nanomedicines, and aid in determination of generic bioequivalence.

1526 Computational Dosimetry Reveals the of Role Particles and Ions in the Toxicity of Soluble Silver Nanoparticles

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When suspended in cell culture media, nanomaterials composed of soluble metals such as silver can undergo dissolution resulting in ion formation and altered particle properties (e.g. mass, morphology, etc.) ultimately modulating cellular dose. Cultured cells are exposed not just to particles, but to a complex, dynamic mixture of particles, free ions, and ion-ligand complexes. Through a variety of nanoparticle dissolution, cellular uptake, and nanoparticle property experiments, a computational model (*In Vitro* Sedimentation, Diffusion, Dissolution, and

Dosimetry Model (ISD3)) was developed to predict particle and ionic dosimetry to cells exposed to silver nanoparticles using a population balance approach. Using three different cell types (bone marrow derived macrophages from wild-type C57BL/6J mice and Scavenger Receptor A deficient mice and RAW 264.7 macrophages), ISD3 accurately predicts nanoparticle dissolution in cell culture media and silver dosimetry for two sizes of silver nanoparticles (20 and 110 nm) and silver ions. Toxicity to those three cell types were measured after exposure to different concentrations of two sizes of silver nanoparticles, and ISD3 was used to predict dosimetrics of nanoparticle exposure conditions. Multiple mixed linear regression model analyses suggest that intracellular nanoparticle surface area was the best predictor of cytotoxicity and viability of all experimental conditions (e.g. cell type, nanoparticle size, etc.). Further experimental results demonstrate that ions formed from nanoparticle dissolution may rapidly complex with ligands reducing toxicity. Overall these results suggest intracellular nanoparticle surface area is the most important determinate of silver nanoparticle toxicity, implicating intracellular dissolution as a potential mechanism. Supported by National Institute of Environmental Health Sciences (NIEHS) U19 ES019544.

1527 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Activity in Low Level Cadmium Exposure

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Exposure to cadmium induces different biochemical responses. In order to investigate the effects of low level cadmium exposure on 3-hydroxy-3-methylglutaryl CoA(HMG-CoA)reductase in the liver, brain and plasma lipid metabolism spectrophotometrically, rats (n = 24; mean weight = 250 g) were exposed to cadmium chloride (100, 200 and 300 ppm) in their drinking water for 12 weeks, while control animals (n = 8) received distilled water for the same period. Exposure to cadmium resulted in highest accumulation of cadmium in the liver from 6.14±0.22 µg Cd/g tissue in control to 54.87±4.86 µg Cd/g tissue in 300ppm dose. Hypocholesterolemia characterized the effect of cadmium with a 50% reduction of control. In the organs examined, highest cadmium dose produced a significant dose-dependent ($p<0.05$) decrease in hepatic cholesterol (45%) and hepatic microsomal cholesterol (42%) concentrations compared to control. There was a non-dose-dependent decrease of 65% in brain cholesterol concentration and dose-dependent of 40% reduction in the brain microsomes of rats exposed to cadmium. Hepatic HMG-CoA reductase activity was reduced by 55% and that of the brain by 39%. Positive associations were observed between hepatic cadmium and HMG-CoA reductase ($r=0.675, p<0.01$); brain cadmium and brain HMG-CoA reductase ($r=0.636, p<0.01$). Spectrophotometric assay of this study revealed that cadmium-induced inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity which indicates that the down-regulation could be responsible for its atherogenic effects.

1528 Involvement of Polyubiquitin-Coding Gene, UBB, in Cadmium Toxicity in Human Proximal Tubular Cells

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Cadmium (Cd) is an environmental contaminant that induces severe clinical symptoms in various tissues including the kidney. Overaccumulated Cd in the kidney damages the proximal tubules. Our previous study demonstrated Cd-induced suppression of the UBE2D gene family, one of the ubiquitin-conjugating enzyme families1). However, the precise role of ubiquitin-coding genes in Cd toxicity remains to be understood. In this study, we investigated the effect of Cd on expression of the ubiquitin-coding genes UBB, UBC, UBA80, and UBA52 in HK-2 human proximal tubular cells. Using MTT assay, it was demonstrated that HK-2 cells treated with 40 µM Cd for 6 hr exhibited 50% cell viability, whereas a 3 h treatment did not induce cytotoxicity. Therefore, we investigated the effect of Cd on expression of 4 Ub-coding genes (UBB, UBC, UBA80, UBA52) with 40 µM Cd for 1, 3, and 6 h by real-time RT-PCR. The 3 h treatment induced expression of UBB, UBC, and UBA80 with the exception of UBA52 compared with non-treated cells. Next, we employed siRNA transfection to target each Ub-coding gene to investigate whether expression changes in Ub-coding genes may affect the sensitivity of HK-2 cells to Cd. Transfection of UBB siRNA markedly decreased UBB mRNA level. Interestingly, UBB siRNA transfection decreased the sensitivity of HK-2 cells to Cd. Transfection of UBA80 siRNA markedly decreased UBA80 mRNA level. However, in contrast to UBB siRNA treatment, UBA80 siRNA-transfected cells exhibited the same Cd sensitivity as control siRNA. Because UBB disruption eliminated Cd toxicity in HK-2 cells, effect of UBB siRNA-treatment on the elevation of ubiquitinated proteins by Cd was examined using western blot analysis. Although the level of ubiquitinated proteins following UBB siRNA treatment alone was the same as for control siRNA treatments, Cd-induced protein ubiquitination in control siRNA treated cells was eliminated by UBB siRNA treatment. These results suggest that UBB is involved in Cd-induced increase of protein ubiquitination, and that accumulation of ubiquitinated proteins through increased UBB expression may contribute to Cd toxicity in HK-2 cells. [This work was partly supported by the Study of the Health Effects of Heavy Metals, organized by the Ministry of the Environment, Japan]
1) Tokumoto, et al., (2011) J. Toxicol. Sci., 36, 191-200.

1529 Low Dose Oral Cadmium Increases Airway Reactivity and Lung Neuronal Gene Expression in Mice

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Inhalation of cadmium (Cd) from occupational exposures or smoking is associated with multiple lung diseases, but less is known concerning pulmonary effects of chronically ingested Cd at levels found in the human diet. Chronic exposure to low doses of Cd, which has a decades-long biological half-life in humans, leads to significant bioaccumulation with unclear toxicological ramifications, particularly for the lung. We exposed mice to 10 mg/L CdCl2 in drinking water for 20 weeks, causing a significant increase in lung Cd burden similar to that of non-occupationally exposed, non-smoking adult humans. Cd-treated mice had significantly increased airway hyperresponsiveness to methacholine challenge compared to controls ($p<0.05$). Using a MoGene ST 2.0 Exon gene array (Affymetrix), we observed that Cd significantly altered 443 genes at $p<0.05$. However, Cd did not elicit increased metallothionein transcripts as expected, a result confirmed by qRT-PCR. To identify the pathways most affected by Cd, transcripts were ranked by abundance and significance and uploaded to the Gene Set Enrichment Analysis applet (Broad Institute) for pathway enrichment. Neuronal receptors were the major inducible targets of Cd, enriching olfactory, cholinergic and serotonergic gene sets at FDR<0.05. Olfactory receptor (Olfr) transcripts, which control chemosensory pathways and airway hyperresposiveness, were the most enriched, and the 5 most significant genes (Olfr 97, 317, 341, 458 and 1416) were validated using qRT-PCR. Furthermore, targeted metabolomics revealed that metabolic precursors of neurotransmitters for nicotinic (choline), glutamatergic (glutamate) and serotonergic (tryptophan) receptors were significantly increased by Cd in lung tissue ($p<0.05$). In conclusion, lung burden from low dose oral Cd exposure was comparable to that of human adults and associated with increased bronchiolar reactivity through neuronal pathways. These results suggest more detailed investigations are needed to elucidate the potential health impacts of dietary Cd, especially on airway hypersensitivity and asthma risk.

1530 Low Level Cadmium Causes Dysglycemia and Decreased Serum Leptin in the db/db Mouse, a Model of Type II Diabetes Mellitus

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Diabetes is a growing worldwide epidemic. Cadmium (Cd) is a ubiquitous environmental pollutant that is associated with hyperglycemia and diabetes. In a recent publication, I show that after 12 weeks of Cd exposure in rats there is an increase in serum levels of glucose-dependent insulinotropic polypeptide (GIP) and a decrease in the level of the satiety hormone, leptin. Based on these observations I conducted a pilot study using db/db mice (type II diabetic mouse model) and lean mice. All animals were given daily subcutaneous injections of Cd at a dose of 0.6 mg/kg/day for two weeks then given no Cd for another two week period. At the end of the study, all animals underwent an oral glucose tolerance test (OGTT), days later serum samples were collected from 5 hour fasted animals. In the OGTT, Cd had the greatest effect on db/db mice, with all animals except one having blood glucose levels of 600 mg/dl or higher 30 min following an oral dose of glucose (2g/kg). At the same 30 min time point, the non-Cd treated db/db mice had an average blood glucose value of 507 ± 19 mg/dl. Db/db mice which have a point mutation in the leptin receptor had a fasting serum leptin value of 271.7 ± 28 (ng/ml) in control vs 170.2 ± 39 in Cd treated animals; $p \leq 0.05$. No significant

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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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- CE Continuing Education Courses
- EC Education-Career Development Sessions
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- HH Historical Highlights Sessions
- IS Informational Sessions
- PL Platform Sessions
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