degradation enzymes, matrix metalloproteinases (MMPs)-2 and tissue inhibitor of MMP-1 in the BALF, which may be involved in the modification of extracellular matrix. At 84 days post exposure, none of the particle treatment groups induced lung inflammation, cellular injury or alteration of hydroxyproline content in lung tissues. These results demonstrated that a thin coating of $aSiO_2$ on CeO_2 protected lungs from CeO_2-induced acute lung toxicity, suggesting that a thin coating of $aSiO_2$ may potentially be used to modify other nanoparticle-induced lung toxicity.

PL 816 Gene Expression Profiling of Human Lung Epithelial Cell Lines Exposed to Manufactured CoO and CeO2 Nanoparticles.

I. Nelissen[1], S. Verstraelen[1], E. Casals[2], S. Remy[1], P. De Boever[1], H. Witters[1] and V. Puntes[2]. *[1]Flemish Institute for Technological Research (VITO NV), Mol, Belgium; [2]Institut Català de Nanotecnologia (ICN), Barcelona, Spain.* Sponsor: P. Hoet.

Exposure to manufactured nanoparticles (NPs) via inhalation can cause adverse human health effects. A transcriptomics study was performed to identify molecules and cellular pathways that are specifically triggered by in vitro exposure of the human bronchial BEAS-2B and alveolar A549 epithelial cell lines to 7-nm CoO and 4-nm CeO2 NPs. We aimed to investigate whether 1) the same lung cell type responds similarly to the NPs, 2) alveolar vs. bronchial epithelial cells respond differently to the same NP, and 3) immunological processes are influenced. Non-cytotoxic exposure concentrations of monodispersed NPs were used. Statistically significant changes in gene expression as compared to solvent-treated cells (median |fold-change|>1.5, $p<0.05$) were evaluated after 3, 6, 10, and 24 hours.
The kinetics of the cell responses induced by the 2 NPs were similar within, but different between the 2 cell models. BEAS-2B cells were found to be more sensitive for NP toxicity, as they showed a higher total number of differentially expressed transcripts (DET) at a 10-fold lower NP-concentration than A549 cells. Hierarchical biclustering of all DET indicated that the transcriptional responses were quite heterogeneous among the 2 cell types and 2 NPs. Between 1% and 14% DET encoding markers involved in immune system processes were observed in the BEAS-2B and A549 cell lines, resp., with the highest fractions observed in BEAS-2B cells. Most of these genes, i.e. ITGB2, TLR6, PAG1, HLA-DRB3, TIRAP, and HLA-A, are involved in immune signalling or yet unassigned pathways. Nanoparticle exposure mainly induced suppression of immune gene transcription, rather than immune stimulation. The AKT1 gene was identified as a possible generic marker of lung epithelial cell-NP interaction.
Our data suggest that CoO- and CeO2-NP give rise to a distinct immunological response in bronchial and alveolar epithelial cells.

PL 817 Mechanistic Insights into the Toxicity of Multiwalled Carbon Nanotubes and Cerium Dioxide Nanoparticles in Primary Human Bronchial Epithelial Cells.

S. Hussain[1], R. Snyder[1], S. M. Anderson[1], J. D. Marshburn[1], A. B. Rice[1], N. J. Walker[2] and S. Garantziotis[1]. *[1]Clinical Research Program, NIEHS (NIEHS)/National Institute of Health (NIH), Durham, NC; [2]DNTP, NIEHS, Research Triangle Park, NC.*

Cerium dioxide nanoparticles (CeO2 NPs) and multi-walled carbon nanotubes (MWCNT) are priority materials for urgent risk assessment due to wide spread industrial, consumer product and environmental utilizations. We aimed at deciphering the impact of CeO2 NPs and MWCNTs on primary human bronchial epithelial cells (BEC) following an ex-vivo exposure. CeO2 NPs and MWCNT suspensions were thoroughly characterized, including using transmission electron microscopy (TEM), dynamic light scattering (DLS), and zeta potential analysis. Cells were then exposed to nanomaterials for 18 - 24 hours and mechanisms of cell injury were studied. TEM revealed that both CeO2 NPs and MWCNTs are internalized by bronchial epithelial cells and are found either in vesicles or free in the cytoplasm. CeO2 NPs fail to elicit a toxic response in BECs at environmentally relevant doses. However, diesel exhaust particles and CeO2 NPs co-exposure leads to significant increase in cytotoxicity. MWCNT exposure in bronchial epithelial cells leads to a time-dependent decrease in viability, increased reactive oxygen species production, NF-κB (p65)/Rel A phosphorylation and nuclear translocation. Moreover, we observed caspase-1 activation and increased numbers of autophagic vesicles in MWCNT-treated cells as compared to control cells. An increase in p62 levels indicated a blockage of autophagic turnover rather than autophagy induction in these cells which was associated with cytoskeletal alterations induced by MWCNT. In conclusion we demonstrate that nanomaterials exposure leads to toxic events in primary human bronchial cells. Moreover, we show that doses of CeO2 NPs and diesel exhaust particles that are innocuous in themselves can result in toxicity when given as a co-exposure.

PL 818 Inflammatory and Free Radical Generation Characteristics of Nano-Cerium Dioxide.

V. C. Minarchick[1], D. W. Porter[2], N. R. Fix[2], S. S. Leonard[2], E. M. Sabolsky[3] and T. R. Nurkiewicz[1]. *[1]Center for Cardiovascular and Respiratory Sciences, West Virginia University, Morgantown, WV; [2]National Institute for Occupational Safety and Health, Morgantown, WV; [3]Mechanical Engineering, West Virginia University, Morgantown, WV.*

Nano-cerium dioxide (CeO_2) possesses the potential for use in human health by protecting against the deleterious effects of ischemia and radiation. However, the literature is polarized about the effects this compound has *in vivo*. Our laboratory has shown that pulmonary nano-CeO_2 impairs arteriolar reactivity 24 hrs post-exposure. The mechanisms of this impairment are currently unknown but may be linked to the free radical scavenging or inflammatory properties of this nanoparticle. The aims of this study were to: 1) thoroughly assess the physical and chemical characteristics of the nano-CeO_2, 2) examine the antioxidant potential of nano-CeO_2 via electron spin resonance (ESR), and 3) assess the pulmonary inflammation in Sprague-Dawley rats 24 hrs post-intratracheal nano-CeO_2 instillation. The primary particle size of the nano-CeO_2 was calculated to ~3 nm (via transmission electron microscopy and surface area measurements). Dynamic light scattering determined the agglomerate size (~80 nm) and x-ray photoelectron spectroscopy determined the valence state of the nano-CeO_2. The ESR measurements indicated that nano-CeO_2 alone did not generate free radicals and in the presence of cells (Raw264.7), nano-CeO_2 quenched the free radicals generated by these cells. Finally, bronchial alveolar lavage from rats instilled with 0, 10, 100 or 400 μg of nano-CeO_2 revealed an increase in polymorphonuclear leukocytes (0.6±0.2, 0.8±0.3, 7.1±0.9, and 10.3±0.9 per 106 cells), and lactate dehydrogenase (90±12, 100±9, 453±33, and 602±32 units/L) but there was no change in albumin. These findings provide evidence that pulmonary inflammation is present after exposure but does not damage to the epithelial/endothelial cellular barrier. Additionally, these nanoparticles are capable of quenching free radicals there by exerting a systemic effect.
NIH-RO1-ES015022 and RC1-ES018274 (TRN), and NSF-1003907 (VCM)

PL 819 Nanoceria Distribution and Biopersistence in Rats Is Not Consistently Affected by Particle Size, Shape, Dose, or Dosing Schedule.

R. A. Yokel[1, 2], R. C. MacPhail[5], J. M. Unrine[3], P. Wu[4] and E. A. Grulke[4]. *[1]Pharmaceutical Sciences, University of Kentucky, Lexington, KY; [2]Graduate Center for Toxicology, University of Kentucky, Lexington, KY; [3]Plant and Soil Sciences, University of Kentucky, Lexington, KY; [4]Chemical and Materials Engineering, University of Kentucky, Lexington, KY; [5]Toxicology Assessment Division, US EPA, Research Triangle Park, NC.*

Background: Nanoceria is a diesel fuel additive, an abrasive in integrated circuit fabrication, and is being developed as an antioxidant therapeutic. Objectives: Determine the influence of nanoceria size, shape, dose, and dosing schedule on its distribution and biopersistence. Methods: Aqueous dispersions of citrate-stabilized cubic or polyhedral ceria and a ceria nanorod (10 x 40 to 600 nm), synthesized and characterized in-house, were iv infused into rats (single infusion of 5 nm @ 11, 56 or 85 mg/kg, 15 nm @ 70 mg/kg, 30 nm @ 6 or 85 mg/kg, 55 nm @ 50 mg/kg, nanorod @ 20 or 50 mg/kg; and 5 nm @ 11 mg/kg for 5 consecutive days). They were terminated 1 h to 90 days later. Controls received vehicle. Multiple organs were weighed and samples collected from multiple sites and blood for cerium determination. Results: The greatest % of the dose was in the liver, spleen and bone marrow; these levels decreased over time only in liver for the 30 nm ceria @ 6 mg/kg, and increased in spleen and bone marrow over time in several cases. There were no consistently significant differences in the % of the dose in the liver or spleen for the different sizes, shapes, doses, or dosing schedules other than tendencies for more nanorod accumulation in the spleen than the 5 nm polyhedral ceria and more nanorod accumulation in the bone marrow than the 5 or 30 nm ceria. Brain nanoceria was low; little to none was in brain parenchyma. Conclusions: Nanoceria, an insoluble metal oxide, was cleared into mononuclear phagocyte system organs in which it persisted for 90 days. Size, shape, dose, and dosing schedule had little effect on its distribution or persistence, suggesting repeated exposure will likely produce accumulation, perhaps reaching a level shown to be toxic after single high-dose administration. Support: US EPA STAR Grant RD-833772.

The Toxicologist

Supplement to *Toxicological Sciences*

52nd Annual Meeting and ToxExpo™

March 10–14, 2013 • San Antonio, Texas

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 132, Issue 1
March 2013

www.toxsci.oxfordjournals.org