intracellular oxidative stress was evaluated by DCFH-DA assay and pro-inflammatory cytokine release (IL-8) was measured by ELISA. 2D-DiGE/MALDI-Tof/Tof approach was used in order to identify possible mechanisms of toxicity. The uptake and localisation of the particles and ions was assessed by Secondary Ion Mass Spectrometry (NanoSIMS50). Contribution of Ag ions to toxicity was also evaluated (ultrafiltration and ICP-MS). Results and discussion: AgNO3 induced a reduction in metabolic activity in a dose dependent manner whereas no reduction was observed in the case of Ag 20nm and 200nm. The presence of mucus showed a protective effect against oxidative stress upon exposure to H2O2. Ag 20nm led to an increase in IL-8 release. Ag was found to be distributed homogenously in the cell with aggregates observed in specific locations in the case of Ag 20nm. Proteomic data revealed that AgNO3 and Ag particles induced an up-regulation of oxidative stress pathways, modulation of cytoskeleton machinery and apoptosis-related proteins. Ag 200nm and 20nm were found to behave in different manner compared to in solution ions. A size dependent effect was observed: 20nm particles seemed to be more effective than Ag 200nm, that were found to be close to the negative control. Conclusions: We described a co-culture model for intestine that is more physiological and relevant for toxicological studies compared to Caco-2 cells alone. Observed differences in effects cannot be attributed solely to ions while the effects were also particle size dependent.

PS 1944 Silica Nanoparticle-Induction of CXCL8 and IL-6 in BEAS-2B Cells via Activation of NF-κB and a p38/TACE/TGF-α/EGFR-Pathway: Role of ROS

M. Refsnes, T. Skuland and M. Låg. *Department of Air Pollution and Noise, Norwegian Institute of Public Health, Oslo, Norway.* Sponsor: B. Granum.

Silica nanoparticles (SiNPs), used in a range of applications, are known to trigger inflammatory responses. We have previously shown that SiNPs of 50 nM size (Si50) induced interleukin (IL)-6 and CXCL8 responses in BEAS-2B cells through combined activation of several pathways, including NF-κB and p38/TACE/TGF-α/EGFR signalling (*Toxicol Appl Pharmacol.* 279(1):76-86). Several studies, but not all, have reported ROS formation to be crucial for SiNP-induced cytokine responses. In the present study, we investigate the role of Si50-induced ROS and scavenger receptors in CXCL8- and IL-6 formation via the NF-κB and p38/TACE/TGF-α/EGFR signalling pathways. Bronchial epithelial cells (BEAS-2B) were exposed to Si50 in DMEM/ F12 without serum. The cells were exposed to N-acetyl cysteine (NAC; 5 mM) for 1 h, or transfected with siRNA against Duox-1, the scavenger receptors (SRB1 and LOX-1) for 24 h, prior to exposing to 200 μg/ml of Si50 . The phosphorylations of p38 and p65 (NF-κB) were analyzed by Western blotting; release of TGF-α, CXCL8 and IL-6 by ELISA, and heme oxygenase expression by Real Time PCR. ROS was measured by spectrofluorometer after staining by DCFDA. Si50 exposure induced formation of ROS, and a time-dependent up-regulation of the mRNA expression of heme oxygenase. The anti-oxidant NAC partially reduced the release of IL-6 and CXCL8, suggesting a role for ROS. Furthermore, NAC-partially reduced phosphorylations of p38 and p65, and the release of TGF-α. Transfection with siRNA against Duox-1 exerted similar effects, but did not inhibit the release of TGF-α. Data on the role of scavenger –receptors in these Si50-induced responses will also be presented. In conclusion, ROS formation seems to participate in the Si50-induced cytokine (IL-6 and CXCL8) release via MAPKinase p38, NF-κB and a cleavage of pro-TGF-α, via NADPH oxidase (Duox-1)-dependent and –independent mechanisms, respectively.

PS 1945 Preliminary Validation Study of a 3D *In Vitro* Inhalation Model, Using Cytokine and Gene Expression Responses of Copper Oxide Nanoparticles

I. M. Kooter[1], M. Grollers-Mulderij[1], E. Duistermaat[2], Y. Staal[2], F. Kuper[1], E. van Someren[1] and E. Schoen[1]. *[1]TNO, Utrecht, Netherlands and [2]TNO Triskelion BV, Zeist, Netherlands.* Sponsor: R. Wouters.

Human 3D airway models are fully differentiated and functional models of the respiratory epithelium. They are cultured at an air-liquid interface (ALI), allowing relevant exposure via air. It is anticipated that these models may predict a more realistic bioavailability of inhaled compounds. To investigate the effects of donor, exposure unit, exposure session and insert, we performed air exposures of copper oxide nanoparticles using the MucilAirTM human 3D bronchial model. MucilAirTM (Epithelix Sàrl) were exposed at ALI conditions in Vitrocell exposure modules to aerosolized CuO (0, 50, 224, 1000 mg/m3) for 1 hour. Donor and exposure module unit were rotated among the four different exposure sessions using a statistical experimental design. Deposition of CuO nanoparticles was 4%. After a 24 hours post-incubation period, exposure to CuO showed a slight but significant LDH response for the highest dose. For inflammation markers MCP-1, IL-8 and IL-6 a dose-response was observed, where this was significant for IL-6. The influence of the parameters 'concentration' is the largest, followed by 'donor', 'unit' and 'session' which are in the same order of magnitude, which is then followed by the parameter 'insert'. Gene expression analyses (using Illumina beadchip (humanHT-12v4)) showed a significant increase in regulated genes (adjusted p-values <0.05) in a concentration dependent way. For the highest dose up to 5852 genes were up- and down regulated. PCA showed clearly distinct groups for 'concentration', as well for 'donor'. Statistical analyses showed that differences in 'concentration' were larger than those among 'donors', while donor differences were more substantial than differences between sessions. We conclude that the MucilAir model can be used to assess the effects of nanoparticles, as long as donor-, session- and chip differences are taken into account of the experimental design and subsequent statistical analyzes.

PS 1946 The Role of Valence State in Cerium Oxide Nanoparticle Toxicity

K. M. Dunnick[1, 2], E. M. Sabolsky[3] and S. S. Leonard[1, 2]. *[1]Health Effects Laboratory Division, NIOSH, Morgantown, WV, [2]Department of Pharmaceutical and Pharmacological Sciences, West Virginia University, Morgantown, WV and [3]Benjamin M. Statler College of Engineering and Mineral Resources, West Virginia University, Morgantown, WV.*

Nanoparticles are part of an emerging field of technology that offers unique manufacturing and engineering properties not easily attainable with micron or larger particles of similar chemical composition. Cerium oxide (CeO_2) nanoparticles are one such material used in a variety of products, including solar cells and gas sensors. Increased industrial production will subsequently lead to additional occupational exposures, making toxicology screenings crucial. Previous toxicology studies have presented conflicting results as to the extent of CeO_2 toxicity, which is theorized to be due to the ability of Ce to exist in both a +3 and +4 valence state. Thus, to study whether valence state is important in CeO_2 toxicity, CeO_2 nanoparticles were doped with gadolinium (Gd) to adjust Ce toward a +3 state. We hypothesized that doping would increase toxicity and decrease antioxidant abilities as a result of increased oxygen vacancies and inhibition of +3 to +4 transition. RLE-6TN rat alveolar epithelial and NR8383 rat alveolar macrophage cells were treated with a range of CeO_2 doses to assess toxicity using an annexin V/ propidium iodide stain, and neither doped nor pure CeO_2 induced toxicity by 24 hrs. Darkfield microscopy was employed to observe nanoparticle-cellular interactions, and within 5 min both pure and doped CeO_2 began to associate with cells. Electron spin resonance, used to assess the effects of CeO_2 on free radical production, showed that with doping, antioxidant potential decreased. Further, Nrf2 activity and downstream antioxidant proteins, such as heme oxygenase-1, were quantified via western blot and immunocytochemistry to elucidate important antioxidant pathways. The results suggest that valence state plays a role in antioxidant potential but a minimal role in cytotoxicity; however, further studies are needed to understand the mechanisms by which CeO_2 valence state affects toxicity.

PS 1947 Proinflammatory Potential of Silica- and Silver-Nanoparticles in Different Epithelial Lung Cell Cultures

M. Låg, T. Skuland, T. H. Nguyen, H. Pham and M. Refsnes. *Department of Air Pollution and Noise, Norwegian Institute of Public Health, Oslo, Norway.* Sponsor: B. Granum.

Amorphous silica nanoparticles (SiNPs) are used in a wide range of applications, including food, cosmetics and medicine. Silver nanoparticles (AgNP) are used in various types of products. Different particle sizes of the same material will inherit different properties and toxicity. The aims of this study were to identify and characterize the acute pro-inflammatory responses of AgNP and different sizes of silica nanoparticles (SiNP) in two different epithelial lung cell lines, and to determine the role of signaling proteins. The human bronchial epithelial cell lines, BEAS-2B and HBEC, were used. The cell lines were grown in LHC-9-medium and substituted with DMEM/F12-medium 1 day prior to particle exposure. The cells were exposed with SiNPs of two sizes; 10 nm (Si10) and 50 nm (Si50), and AgNP of 20 nm (Ag20). The expression and release of the cytokines interleukin (IL)-6, CXCL8 and RANTES (CCL5) were analyzed by real time PCR and ELISA. LDH release, used as a cytotoxic marker, was measured by a colometric assay. Involvement of signaling proteins were studied by using chemical inhibitors, gene silencing (siRNA) together with activation/ phosphorylation of the signaling proteins (MAPKs and NF-κB) by Western blotting. The pro-inflammatory responses varied between the different NPs. The expression and releases of IL-6, CXCL8 and CCL5 were much higher for Si10 than for Si50. Although the Ag20 had a size between the two SiNPs, the cytokine responses were even lower than for Si50.The cytokine responses were similar in the two lung cell cultures. For both SiNPs the responses seemed to be mediated partly through epidermal growth factor receptor (EGFR) and the MAPKs p38 and JNK. Thus, Si10 and Si50 are suggested to mediate cytokine responses by the same

The Toxicologist

Supplement to *Toxicological Sciences*

54th Annual Meeting and ToxExpo™

March 22–26, 2015 • San Diego, California

The Official Journal of the Society of Toxicology

OXFORD UNIVERSITY PRESS

ISSN 1096-6080
Volume 144, Issue 1
March 2015

www.toxsci.oxfordjournals.org

SOT | Society of Toxicology

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

www.toxicology.org