

profiles of single nanoparticle exposures, but in order to effectively cope with health and environmental concerns, we must also know how these sole nanoparticles interact with other particles in biological and ecological environments.

**PS 888** GENERATION OF REACTIVE OXYGEN SPECIES BY SILICON NANOWIRES.

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Silicon nanowires (NW) are anisotropic crystals with semi-conductor capabilities and are currently being utilized in the production of biosensors, gas sensors, and field electric transistors. Because of their composition and large length to diameter ratio, silicon NW-induced toxicity may differ from other forms of nanoparticles. A variety of different forms of nanoparticles have been shown to produce toxic effects in cellular and animal models through the induction of oxidative stress. The potential for oxidative damage after exposure to silicon NW has not been investigated. The goal of this study was to assess the generation of free radicals by silicon NW. Electron spin resonance (ESR) was used to monitor hydroxyl radical production in an acellular system by measuring Fenton-like reactions in the presence of hydrogen peroxide. In addition, the ability to produce radicals after cellular exposure was also determined by ESR. These measures of particle surface reactivity of silicon NW (20 nm diameter x 10 µm length) at two different concentrations (0.2 mg/ml or 0.1 mg/ml), were compared to those of titanium dioxide (TiO<sub>2</sub>) NW (0.1 mg/ml or 0.05 mg/ml), amosite asbestos fibers (0.2 mg/ml or 0.1 mg/ml), and lead chromate particles (PbCrO<sub>4</sub>) as a positive control (0.5 mg/ml). For *in vitro* studies, two cell types were used to measure reactive oxygen species (ROS) production, primary rat macrophages harvested from the lungs of male Sprague-Dawley rats and the RAW 264.7 macrophage cell line. ESR results showed no presence of hydroxyl radical or ROS production with silicon NW, in either the acellular or cellular systems. Hydroxyl radicals were observed in PbCrO<sub>4</sub> and amosite asbestos. However, radicals were not seen in TiO<sub>2</sub> NW. These findings indicate that silicon NW may not induce a significant increase in oxidative stress and associated damage.

**PS 889** UNANTICIPATED BREAKDOWN PRODUCTS FORMED USING DMSO AS A SOLVENT TO STUDY THE AGGREGATION EFFECTS OF TiO<sub>2</sub> ON MARINE MICROORGANISMS.

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In contrast to chemical stressors, most nanomaterials have a tendency to aggregate in aqueous environments. The aggregation behavior and solubility of suspensions of these materials in aqueous media are typically mediated by pH, ionic strength, and the presence of organic solvents as well as by physical mixing techniques such as ultrasonication. It is herein shown that the use of sonication to decrease aggregation and increase the suspension of TiO<sub>2</sub> (rutile) in DMSO results in the degradation of DMSO and formation of sulfonic and sulfonic acids. The concentrations of sulfonic and sulfonic acids in DMSO as shown by ion chromatography and features in FTIR spectra, increase with sonication time both in the presence and absence of TiO<sub>2</sub> (rutile). The presence of TiO<sub>2</sub> increases the concentration of these breakdown products over a 3 hr time frame. Due to the toxicity of sulfonic and sulfonic acids, resulting from the sonication, DMSO became unsuitable as a solvent to study the effects of aggregation of TiO<sub>2</sub> on the marine organism *Vibrio fischeri*. It is further suggested that the use of sonication to suspend nanomaterials in DMSO may produce unanticipated results for toxicity studies in other organisms.

**PS 890** CYTOKINES EXPRESSION *IN VITRO* AFTER EXPOSURE OF CO-CULTURES TO NANOSIZED MONODISPERSE SILICA PARTICLES.

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We have previously shown that the cytotoxicity of monodisperse amorphous silica nanoparticles in human endothelial cells (EAHY926 cell line) was strongly related to particle size. The aim of the present study was to test the influence of nanoparticles size on the cytokines expression using co-cultures of pulmonary epithelial cells with macrophages and endothelium cells using a two compartment system.

We used amorphous (monodisperse) spherical silica nanoparticles with a diameter ranging from 2, 16, 60, 104 to 340 nm. Particles were incubated with epithelial cells and macrophages (A549 + THP-1) at a concentration of 5 µg/cm<sup>2</sup> cell cultures surface or at 10 cm<sup>2</sup> particle surface area/cm<sup>2</sup> for 24 h whether or not in the presence of confluent EAHY926 cells in an insert introduced above the biculture after 12 h. At 24 h, supernatants were recovered and TNFalpha, IL-6, IL-8, MIP-1alpha and MIP-1beta were measured by means of a bead assay.

Quantification using the FACS array system showed significant increases for TNFalpha, IL-6, IL-8, MIP-1alpha for the 2 nm particles in all conditions tested. The larger particles did not induce cytokine expression at 5 µg/cm<sup>2</sup>, but at dosing 10 cm<sup>2</sup> particle surface area/cm<sup>2</sup> (and thus a large mass) also the larger particles induced a response. In the bicultures, TNFalpha, IL-8 and IL-6 were most prominent, while in the EAHY926 cells also MIP-1beta was significant increased. This work clearly shows the role of size and surface area in response to nano-silica. Work was financed by the Belgian Ministry of Scientific Policy in the frame of the 'Science for sustainable development' programme (S<sup>2</sup>NANO project, contract number SD/HE/02A).

**PS 891** *IN VITRO* TRANSLOCATION OF QUANTUM DOTS AND INFLUENCE OF OXIDATIVE STRESS.

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**BACKGROUND:** Translocation of inhaled nanoparticles to the circulation has been demonstrated *in vivo*. However, the interaction of nanoparticles with the lung epithelium and how they translocate through the cell layer is not understood.

**AIM & METHODS:** In this study we investigated, *in vitro*, the translocation of nano-sized quantum dots (QDs) (25 pmol/ml) through a tight monolayer of primary rat type II pneumocytes. The influence of surface charge on translocation was examined by using non-functionalized QDs, amine-QDs and carboxyl-QDs. The interaction between the nanoparticles and the lung epithelium were monitored by repeatedly measuring the "transepithelial electrical resistance" (TEER) and by examining the cell layer with confocal microscopy. The effect of oxidative stress was tested by culturing the cells together with tert-butyl hydroperoxide and the antioxidant N-Acetyl-L-cysteine was used to assess the role of particle-mediated oxidative stress.

**RESULTS:** Using the different types of QDs, no translocation through a tight monolayer of primary rat type II pneumocytes was observed. In general, an increase in TEER was found after incubation with QDs. A condition of low oxidative stress did not enhance translocation. In contrast, conditions of high oxidative stress with disruption of the cell layer, as shown in a decreased TEER, resulted in 30% translocation, regardless of the type of QDs.

**CONCLUSIONS:** No translocation of QDs was found through a tight monolayer of primary rat type II pneumocytes, regardless of the QDs surface charge. In addition, QDs did not impair the barrier function of the epithelial cells. In conditions of high oxidative stress with disruption of the cell-cell barrier, 30% translocation was observed.

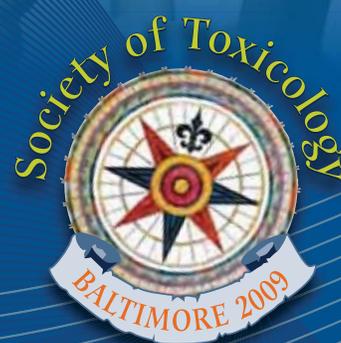
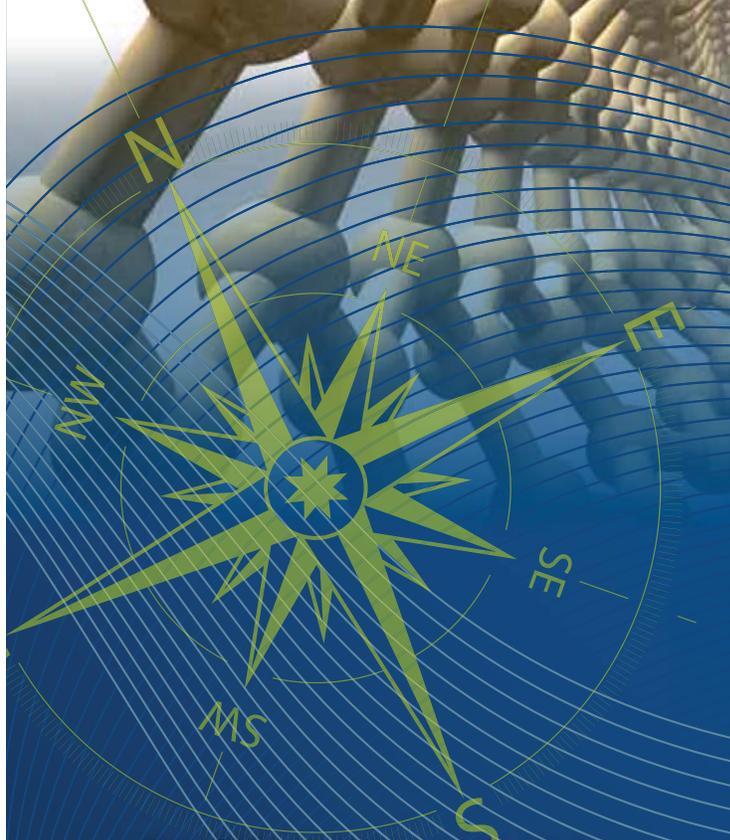
**PS 892** MECHANISTIC APPROACH TO COMPARE TOXICITY OF THREE UNIQUE NANO-SIZED METAL COLLOIDAL SUSPENSIONS TO LIVER CELL CULTURE SYSTEMS.

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Nano-sized metal colloids are studied for both industrial and medicinal purposes. To resolve some of the outstanding toxicity concerns, we are utilizing a model that more accurately mimics the response of the liver in an *ex vivo* cell culture system, namely co-cultures of primary hepatocytes, Kupffer's cells, and lymphocytes isolated from C57BL6 mice. The manufactured inorganic metal nanomaterials include colloidal silver (Ag), copper (Cu), and nickel (Ni). The sizes, characterized in the dry state using BET surface area analyses and in the wet phase using dynamic light scattering and electron microscopy, vary with time, temperature, and media. Further, the pH of each suspension changes over time. We also found metal ions leaching from each material via atomic emission spectroscopy. These dynamic physico-chemical properties influence the biological response in two distinct pathways: a heavy metal cationic effect versus a nano-size effect. Cells were exposed to the three metal nanomaterials, independently. Post-exposure time points ranged from 4 hr to 1 wk. Colorimetric cytotoxicity was measured using MTT and resazurin dyes. Inflammatory response was determined by cytokine profiles (TNF-alpha and IL-6). A stable PXR-transfected luciferase reporter cell line (JBC, Gu) was used to test the effects of the nanomaterials on the pregnane X transcription activity. Nano colloidal Ag, Cu, and Ni treatment enhanced the PXR ligand RIF-induced reporter gene activity, suggesting an alteration of the xenobiotic metabolism. The mechanism for the interaction will be further analyzed. Results from cells ex-

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