

## 1510 NANOPARTICLES SELECTIVELY STIMULATE NADPH OXIDASE IN RAT PULMONARY CELL LINES.

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Nanomaterials are being identified for a number of applications ranging from cosmetics to circuitry. With the increase in production and use comes an increase in the potential for human and environmental exposures which when combined with an uncharacterized nanoparticle hazard presents a potential increase in risk. At the current time the mechanism of toxicity of engineered nanoparticles is not clearly understood. There has been some research suggesting that nanoparticles are capable of eliciting their damage via oxidative damage through the production of reactive oxygen species. This study addresses a possible mechanism of this toxicity; namely through induction of the NADPH oxidase complex. Rat derived pulmonary epithelial and endothelial cell lines were cultured and exposed to copper, titanium dioxide, silver and aluminum nanoparticles in the presence of a cell impermeant fluorescent dye (Amplex Red®) to assess the extracellular oxidant production following nanoparticle stimulation. Alveolar type I like and endothelial cell lines react to particle stimulation by increased production of oxidants while alveolar type II cells showed minimal response. To elucidate the role of NADPH oxidase in this system cells were incubated with pharmacologic inhibitors of NADPH oxidase or with agents known to disrupt the assembly of an active complex through disruption on membrane lipid rafts. We can conclude that nanoparticles possess an intrinsic radical producing activity which appears to be supplemented by cellular ROS emanating from the NADPH oxidase complex. These effects varied with both particle and cell type, underlining the importance of considering the system used to assess nanoparticle toxicity.

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## 1511 UPTAKE OF FINE AND ULTRA-FINE POLYSTYRENE LATEX BEADS IN A549 CELLS.

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Human exposure to nanomaterials is a potential public health concern due to their dramatic increased production and use in consumer products. Emerging research has demonstrated that nanoparticles are transported into cells; however, the consequences of this uptake as well as the mechanism of cellular uptake have not been fully elucidated. In this study, we used a type-II-like lung alveolar cell line (A549) to examine the mechanisms of uptake of ultrafine (26 nm) and fine size (250 nm) fluorescent polystyrene latex (PSL) beads. Following a 20 min in vitro exposure, flow cytometry and confocal microscopy reveal that uptake of the ultra-fine particles occurs via both temperature-dependent and temperature-independent pathways and that cellular uptake involves actin. Uptake of the fine particles is temperature-dependent, and involves actin and microtubule proteins. Clathrin and caveolae-dependent mechanisms do not appear to play a role in the internalization of either particle as pretreatment of the cells with chlorpromazine or low potassium and filipin, respectively, had no effect on uptake. Taken together, these data suggest that different endocytic processes mediated the uptake of the ultra-fine and fine PSL beads in A549 cells. These findings improve our understanding of the movement of nanomaterials across cell membranes and may provide insights into how these processes heighten their toxicity or improve their drug delivery capability.

## 1512 NANOPARTICLE DEPOSITION EFFICIENCY IN RAT AND HUMAN NASAL REPLICAS.

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Studies of particulate morbidity and mortality have suggested that airborne ultra-fine particles (< 0.1 µm) may pose a greater hazard primarily due to their small size and potential to deposit in the deep lung. With increasing production of nanoparticles (materials deliberately produced in the size range of 1 to 100 nm), there is concern over the health effects of inhaled engineered nanoparticles. Measurements were made of the deposition efficiency of nanoparticles in replicas of one F344 rat and four healthy human nasal airways. Monodisperse nanoparticles of sucrose or diethylhexyl sebacate (DEHS) in the size range from 5 to 100 nm were produced using an electro-spray aerosol generator (Model 3480, TSI, Inc., Shoreview, MN) or a nebulizer (one-jet Collison nebulizer CN241, BGI, Inc, Waltham, MA) with electrostatic classifier (Model 3080, TSI, Inc., Shoreview, MN). The aerosol was passed through a nasal replica in the inspiratory direction at a constant flow rate. The concentration of the nanoparticles was measured upstream and down stream of the

nose model using a scanning mobility particle sizer (SMPS, Model 3934 or Model 3036, TSI, Inc., Shoreview, MN). The deposition fraction was determined from the difference in particle concentration between the upstream and downstream measurements. In general, for all of the replicas (1 F344 rat and 4 humans), the deposition efficiency increased as particle size decreased. For the 4 human nasal replicas, the deposition efficiency did not appear to depend on flow rate or nasal geometry. These results were consistent with diffusivity playing a major role in the deposition of particles in the nanometer size range. The results from this study have been used in computer models of particle deposition in the respiratory tract (See poster by B. Asgharian). This work was funded by US EPA STAR Grant #RD832531 and the Long Range Research Initiative of the American Chemistry Council.

## 1513 EFFECTS OF CERIUM OXIDE ON RAT PRIMARY ALVEOLAR MACROPHAGES.

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Diesel emissions pose health concerns in both environmental and occupational settings. The use of cerium as a fuel-borne catalyst lowers emission of carbonaceous diesel exhaust particles (DEP) and their hydrocarbon content, but results in the emission of ultrafine and fine cerium oxide particles. Due to its nanoparticle size, cerium oxide emission is a potential cause of health concern. Cerium oxide is a thermally stable strong oxidant and is capable of mediating cell-derived nitric oxide levels; thus, it may have a strong effect on alveolar macrophage (AM)-mediated immune/inflammatory responses. The objective of this study is to characterize the in vitro dose-dependent effects of cerium oxide (1.25 to 50 µg/ml) on rat AM functions in the absence or presence of DEP (50 or 100 µg/ml). AM were isolated from Sprague Dawley rats via bronchoalveolar lavage, cultured with cerium oxide, DEP or their combination for 24 h. Intracellular oxidants and mitochondrial damage of AM were determined using confocal microscopy. The particle-induced cytotoxicity and pro-inflammatory cytokine production by AM were monitored as lactate dehydrogenase (LDH) activity or the level of TNF-α and IL-12 in the culture media, respectively. The results show that cerium oxide induced LDH release, significantly blocked resting and LPS-induced nitric oxide and IL-12 production in a dose-dependent manner, but did not induce intracellular superoxide generation, mitochondria damage, or TNF-α secretion. Cerium oxide did not affect DEP-induced intracellular ROS or mitochondria damage, but significantly reduced DEP-induced IL-12, but not TNF-α, production at rest or in response to LPS. This study shows that direct interaction of cerium oxide with AM results in dose-dependent cellular damage and suppression of macrophage responses to LPS or DEP in the production of nitric oxide and IL-12, thus indicating a potential suppressive effect by cerium oxide on the innate and cell-mediated immunity against respiratory infection.

## 1514 METHODOLOGY FOR GENERATING NANOPARTICLE AEROSOLS- FROM BENCHTOP TO INHALATION LABORATORY.

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Issues surrounding occupational safety of workers exposed to nano-sized particles and materials are becoming increasingly pertinent in nanotechnology today. We have developed a novel approach to generating and characterizing a nanoparticle aerosol stream appropriate for testing, both in the workplace environment as well as for animal toxicity exposures. Although other methods are known to produce nano particulate matter, a limited number of techniques are available to synthesize fully characterized particle aerosols in the nano-size regime with precise control over size, size distribution, and concentration. The work described herein presents a proven method of producing amorphous nano-sized SiO<sub>2</sub> particles via an aerosol nanoparticle reactor. Other particles consisting of different chemical composition can be synthesized as well (e.g., citric acid, TiO<sub>2</sub>, NaCl). The reactor is capable of synthesizing particles ranging d<sub>50</sub> = 10 – 70 nm at concentrations ranging 10E<sup>4</sup> – 10E<sup>7</sup> particles/cm<sup>3</sup>. Characterization instrumentation in-line with the generation system allows for crystallinity (XRD), size and size distribution (DMA), and concentration (AE) measurements to be made in situ and upstream of the animal exposure chamber. This un-aggregated particle generation methodology enables subsequent testing with a continuous, stable supply of aerosol nanoparticles to facilitate inhalation toxicological profiles of animals exposed to aerosolized nanoparticle systems. This system was utilized in a short-term inhalation study with rats exposed to uncoated as-synthesized, amorphous SiO<sub>2</sub> in two different nanoparticle size range populations (i.e. number distributions centered at d<sub>50</sub> = 30 nm and d<sub>50</sub> = 70 nm). The pulmonary toxicity results are described in a companion presentation.

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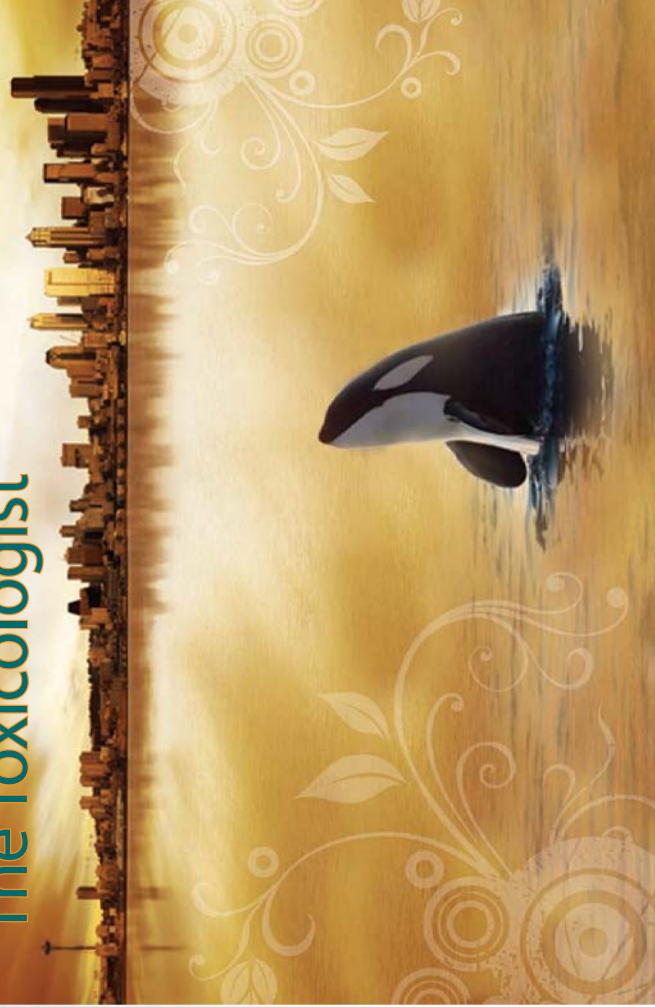
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