while the fine-TiO2-induced changes were minimal. Phosphorylation of ERK1/2 occurred following 10 min exposure to higher doses of ultrafine TiO2 (above 25 μg/ml). Similarly, ultrafine TiO2 exposure significantly enhanced TNFα and MIP-2 secretion in a dose-dependent manner and its potency was higher than fine TiO2. These findings suggest that at relatively low doses of the particles, ultrafine TiO2 has greater biological activity associated with oxidative stress, such as ROS generation, ERK 1/2 activation, and proinflammatory mediator secretion, in RAW 264.7 macrophages than fine TiO2.

1487 EVALUATION OF *IN VITRO* CELL CULTURE SYSTEMS TO ACCURATELY PREDICT THE PULMONARY TOXICITY OF INHALED OR INSTILLED FINE OR NANO ZINC OXIDE PARTICLES.

D. B. Warheit, M. B. Golt, C. M. Sayes and K. L. Reed. *DuPont Haskell Laboratory, Newark, DE.*

Previous studies have reported little correlation between the relative toxicity of particle-types when comparing lung toxicity rankings following in vivo exposures compared to in vitro, cell culture exposures. This study was designed to assess the capacity of in vitro screening assays to predict in vivo pulmonary toxicity of fine (111 nm, 9.6 m2/g) or nanoscale zinc oxide (90 nm, 12 m2/g) particle-types in rats. Rats were exposed by intratracheal instillation to 1 or 5 mg/kg of nanoscale or fine size zinc oxide particle-types. For the inhalation studies, rats were exposed to aerosols of 25 or 50 mg/m3 for 1 or 3 hours. For both types of in vivo studies, lung inflammation, cytotoxicity, cell proliferative and histopathological endpoints were assessed at several time points postexposure.
For the in vitro component of the study, three different culture conditions were utilized. Cultures of 1) rat lung epithelial cells, 2) primary alveolar macrophages, as well as 3) alveolar macrophage – L2 lung epithelial cell co-cultures were incubated with fine or nano ZnO, and the culture fluids were evaluated for cytotoxicity endpoints (LDH, MTT) as well as inflammatory cytokines (MIP-2, TNF-α, and Interleukin-6) at different time periods – 1 hr, 4 hrs, 24 hrs or 48 hrs.
In vivo exposures to instilled or inhaled fine or nanoscale zinc oxide produced a "metal fume fever" type of response, with extended but transient lung inflammatory or cytotoxic responses which were resolved after a few days postexposure. Alternatively, in vitro exposures to fine or nanoscale ZnO produced cytotoxic responses at 4 or 24 hrs but not 1 or 48 hrs incubation in L2 cells and co-cultures, but not in macrophages. Cytokine generation (MIP-2, TNF-α, and Interleukin-6) in ZnO-exposed cells was not significantly different from controls at any dose or time period. To summarize, the comparisons of in vivo and in vitro toxicity measurements demonstrated little correlation, particularly when considering many of the variables assessed in this study.

1488 ASSESSING THE PULMONARY IMPACTS OF TWO SELECTED AMORPHOUS SILICA NANOPARTICLE SIZE RANGES IN RATS FOLLOWING INHALATION EXPOSURES.

C. M. Sayes[1], M. L. Ostraat[2], K. L. Reed[1] and D. B. Warheit[1]. *[1]DuPont Haskell Laboratory, Newark, DE and [2]DuPont Central Research and Development, Wilmington, DE.*

Few data have been generated on the pulmonary toxicity of inhaled nanoparticles per se. This research was designed to systematically evaluate the role of nanoparticle size on pulmonary toxicity parameters in rats exposed by inhalation to smaller (30 nm) vs. larger (70 nm) sized silica nanoparticles. The experimental design was divided into four parts: 1) nanoparticle generation, 2)nanoparticle characterization, 3) stability over time, and 4) mammalian pulmonary hazard effects. To initiate this experimentation, aerosol nanoparticle test materials of untreated, as-synthesized, amorphous SiO2 in two different nanoparticle size range populations (i.e. number distributions centered at d50 = 30 nm and d50 = 70 nm) were developed. Each of the size populations was synthesized in situ and upstream of the inhalation apparatus. The aerosolized nanoparticle populations were tested for stability over time by measuring chamber temperatures and humidity. Groups of 5 animals were exposed to 1.5 × 10E7 particles/cm3 for either 1 × 5 hour period or for 3 × 5 hour periods over 3 consecutive days to establish a dose-response relationship at each post-exposure time-point (24 hrs, 1 wk, and 1 mo). We also conducted a flow cytometric assessment of induction of micronuclei in peripheral blood samples. During the nanoparticle aerosol exposures to rats, particle characterization data was collected at t=0, 1, 2.5, 4, and 5 hours. Characterization results demonstrated precise size populations with 5% size distributions; chemical composition (SiO2), surface charge (-30 mV), and amorphous structure remained identical over the exposure duration. Subsequent post-exposure pulmonary toxicity results (including BAL fluid inflammatory and cytotoxicity endpoints) demonstrated little difference in response to the two different sized nanoparticle-types when comparing exposures to the two different nanoparticle size populations or to sham-exposed air controls. Genotoxicity and lung tissue analysis studies are on-going.

1489 MECHANISMS OF NANODIAMOND PARTICLE INDUCED IL-8 EXPRESSION IN HUMAN AIRWAY EPITHELIAL CELLS.

R. Silbajoris[1], M. Huang[3], W. Cheng[5], L. A. Dailey[1], R. K. Saxena[4], P. A. Bromberg[2] and J. M. Samet[1]. *[1]NHEERL, U.S. EPA, Chapel Hill, NC, [2]CEMALB, UNC, Chapel Hill, NC, [3]Duke University, Durham, NC, [4]School of Life Sciences, Jawaharlal Nehru University, New Delhi, India and [5]Environmental Sciences and Engineering, UNC, Chapel Hill, NC.* Sponsor: M. Madden.

Nanodiamond particles (NDP) prepared by detonation under confined conditions have a number of industrial and analytical applications. Previous *in vitro* studies have reported NDP to be biologically inert with negligible cytotoxicity, implying that they are potentially suitable for biomedical applications such as drug delivery. Separate studies have shown that elemental carbon particles that simulate the carbonaceous core of combustion derived airborne particulate matter can induce inflammatory responses in the lung through the generation of reactive oxygen species. To assess the respiratory effects of exposure to NDP, we examined its effects on IL-8 expression by human airway epithelial cells (HAEC) *in vitro*. Four hour exposures of HAEC to 66 μg/ml NDP (average particle diameter of 5 nm and surface area ~300 m^2/g) resulted in IL-8 mRNA increases up to 100-fold over resting levels and was accompanied by up to 13-fold increases in IL-8 protein levels in the media. Transfections with IL-8 promoter constructs showed that exposure to NDP increases IL-8 transcriptional activity. Separate experiments indicated that HAEC avidly take up NDP and that this uptake can be blocked with cytochalasin D. Furthermore, cytochalasin D also abrogated NDP induced IL-8 mRNA levels. NDP-induced IL-8 expression was blunted by overexpression of catalase, and to a lesser extent SOD, in HAEC. We conclude that NDP induce IL-8 expression via a transcriptional mechanism that requires particle uptake and involves the formation of reactive oxygen species. These data are evidence of an inflammatory response to NDP exposure in human lung cells. THIS ABSTRACT OF A PROPOSED PRESENTATION DOES NOT NECESSARILY REFLECT EPA POLICY.

1490 GOLD NANOPARTICLES INDUCE OXIDATIVE DAMAGE IN LUNG FIBROBLASTS *IN VITRO*.

L. Yung[1], J. J. Li[2], L. Zou[3], D. Hartono[1], C. Ong[3] and B. Bay[2]. *[1]Chemical & Biomolecular Engineering, National University of Singapore, Singapore, Singapore, [2]Anatomy, National University of Singapore, Singapore, Singapore and [3]Community, Occupational and Family Medicine, National University of Singapore, Singapore, Singapore.* Sponsor: U. Boelsterli.

Scope: Nanomaterials are found to have many uses and potential applications in the fields of biology and medicine. Gold nanoparticles (AuNPs) in particular are being developed as novel gene and drug delivery agents as well as various imaging systems due to due to the inertness of bulk gold. Recent research findings have brought to light concerns over the safety of nanomaterials and long-term adverse effect of their use. In this study, we exposed AuNPs to lung fibroblast in vitro and studied the adverse effects of the nanoparticles to the cells.
Experimental procedures: MRC-5 human lung fibroblast cell line was exposed to 20 nm AuNPs at three concentration (0.1, 0.5, and 1 nmol particle per liter) for 24, 48, and 72 hours. Cell proliferation and viability were evaluated using trypan blue exclusion. TEM was used to image AuNPs internalized by the fibroblasts. To ascertain if AuNPs induce oxidative DNA damage, we analyzed the quantity of 8 hydroxydeoxyguanosine (8OHdG) which causes mutagenicity through G-C to T-A transversions resulting in 8OHdG-A mispaired bases.
Results: There were no visible alterations in the cell morphology between the treated and control (media only without AuNPs) groups at different time points, however, cell proliferation showed a significant difference in the total number of cells at 72 hours following AuNP treatment. AuNPs taken up by the fibroblasts showed up as dark dense clusters under TEM. They mostly gathered in clusters inside cellular vesicles. In some cases, scattered AuNPs were found in the cytosol. DNA damage analysis showed that 8OHdG/106dG value in 1 nmol particle concentration AuNP treated cells was significantly higher than the control, implying oxidative DNA damage when the cells were exposed to higher concentration of AuNPs.
Conclusions: AuNPs may induce some degree of cytotoxicity in human lung fibroblasts via inhibiting cell proliferation and oxidative damage.

1491 PULMONARY RESPONSE TO INTRATRACHEAL INSTILLATION OF FINE OR ULTRAFINE CARBON BLACK OR TITANIUM DIOXIDE: ROLE OF SURFACE AREA.

T. M. Sager[1, 2], D. Porter[1, 2] and V. Castranova[1, 2]. *[1]NIOSH, Morgantown, WV and [2]WVU School of Medicine, Morgantown, WV.*

Nanparticles are characterized by having a high surface area per mass. Therefore particle surface area may play an important role in determining the biological activity of nanoparticles. However problems arise because nanoparticles tend to agglomerate into μm sized particles when suspended in PBS.

Our previous studies found that such agglomeration in PBS results in inaccurate dose delivery this leads to misinterpretation of toxicity of the particulate being assessed. We found that acellular BALF is effective in dispersing nanoparticles without masking the particles biological surface activity. After establishing an accurate protocol for dispersing nanosized particles, assessment of the inflammatory and cytotoxic potential of ultrafine and fine carbon black and TiO2 was conducted. Fisher rats were exposed by intratracheal instillation to varying doses of ultrafine and fine carbon black or TiO2. At 1,7, or 42 days post-exposure, inflammatory and cytotoxic potential of each particle type was compared on both an equal mass dosage (mg/rat) as well as an equal surface area dosage (cm2 of particles per cm2 of alveolar epithelium). The findings of the study show that on an equal mass basis the ultrafine particles were significantly more inflammogenic and cytotoxic than the fine sized particles. However, when doses were equalized based on surface area of particles given, the ultrafine particles were only slightly more inflammogenic and cytotoxic when compared to the fine sized particles. It was also noted that at 1 day post-exposure, with a dose equalized to surface area, the carbon black particles and the TiO2 particles exhibited similar inflammogenic potential. Over the post-exposure time course of 42 days pulmonary toxicity of both the ultrafine and fine particles tended to resolve. However at 42 days post-exposure the TiO2 was significantly more potent than the carbon black particles. In conclusion this study suggests that surface area of particles may be a more accurate dose metric for pulmonary toxicity studies than mass of particles.

1492 ENGINEERED TITANIUM DIOXIDE NANOWIRE TOXICITY *IN VITRO* AND *IN VIVO*.

D. W. Porter[1], A. Holian[3], K. Sriram[1], N. Wu[2], M. Wolfarth[1], R. Hamilton[3] and M. Buford[3]. *[1]Health Effects Laboratory Division, NIOSH, Morgantown, WV, [2]Mechanical & Aerospace Engineering, West Virginia University, Morgantown, WV and [3]Center for Environmental Health Sciences, University of Montana, Missoula, MT.*

While the application and benefits of manufacturing nanowires is highly promising, their adverse effects have not been fully investigated. For these studies, TiO_2 nanowires (anatase, diameter=80 nm, length=20-25 μm) were synthesized using sol-gel process directed by a porous anodic aluminum oxide template. We conducted in vitro studies using alveolar macrophages isolated from both C57Bl/6 and Balb/c mice assessing toxicity with 4 hr suspension incubations by trypan blue exclusion and apoptosis using Cell Death ELISA. TiO_2 nanospheres caused no toxicity or apoptosis up to 200 μg/ml. In contrast, TiO_2 nanowires caused significant and marked dose-dependent toxicity and increase in apoptosis. Furthermore, TiO_2 nanowires, but not nanospheres increased alveolar macrophage antigen presenting activity (using ovalbumin and T cells from DO11.10 mice) to similar extents as the potent particle crystalline silica. For in vivo studies we exposed C57Bl/6 mice by pharyngeal aspiration to TiO_2 nanowires (0-80 μg/mouse) and examined lung and brain responses at one day post-exposure. In the lung, exposure to TiO_2 nanowires induced dose-dependent increases in the expression of the inflammatory mediators TNF-α (1.8- to 5-fold), MIP-2 (4- to 33-fold) and CCL2 (7- to 30-fold). In the brain, pulmonary exposure to TiO_2 nanowires induced expression of the endothelial cell adhesion molecule E-selectin in olfactory bulb (4-6 fold), suggestive of altered blood brain barrier (BBB) permeability. Unlike the dose-dependent pulmonary effects, the neural responses were elicited only by higher doses of the nanowires. Whether the BBB changes observed are a consequence of the translocation of these nanoparticles to the brain or a systemic inflammatory response remains to be investigated. Taken together, the data suggest that exposure to TiO_2 nanowires may result in adverse health outcomes. Supported in part by grants ES-015497 and RR-017670 (A. Holian, PI)

1493 COMPARATIVE PULMONARY RESPONSE TO INHALED MULTIWALLED CARBON NANOTUBES, CARBON BLACK, AND ALPHA QUARTZ.

J. Pauluhn. *Toxicology, Bayer HealthCare, Wuppertal, Germany.*

Baytubes® is a Multiwalled Carbon Nanotubes (BT-CNT) material that consists of large agglomerates. To make inhalation testing possible micronization was necessary. Wistar rats were exposed for 1x6 h by inhalation to aerosolized crystalline quartz(Q), carbon black (Printex® 90, P90; BET: 300 m2/g), and two concentrations of BT-CNT (BET: 250 m2/g), at 248, 229, 11, and 241 mg/m3, respectively. The MMAD was for Q, P90, and BT-CNT low/high 2.3, 2.0, 2.9/2.2 um (GSD 1.8-2.6). Rats (6/serial sacrifice) were examined on postexposure days 7, 28, and 90. Inflammatory endpoints were determined in bronchoalveolar lavage (BAL). The concentration of Cobalt in lungs, lung-associated lymph nodes (LALN), brain, kidneys, testes, and liver were analyzed as a translocation marker for BT-CNT. The H&E and Sirius Red stained lung sections of rats exposed to BT-CNT were examined by histopathology (days 28 and 90). In Q-exposed rats the LALN weights increased time-dependently. By contrast, those of CB and BT-CNT exposed rats declined to the level of the control. BAL-protein, LDH, and –collagen showed a similar trend. The influx of neutrophilic granulocytes (PMNs) was most pronounced in Q-exposed rats. At 11 mg BT-CNT/m3 (day 90), most endpoints in BAL were similar to the control or CB groups. Histopathology revealed an increased cellularity in the bronchioloalveolar region (focal septal thickening and fibrosis) at 241 mg/m3. The distinct time-related exacerbation observed in Q-exposed rats did not occur in BT-CNT or P90-exposed rats. Despite a concentration-dependent increase of Co in lung tissue translocation to extrapulmonary organs did not occur. The clearance of Co during the 3 months postexposure period was 96% and 43% at 11 and 241 mg BT-CNT/m3, respectively. In summary, at the end of the 3-months postexposure period, the time-related exacerbation observed following exposure to Q was not seen in rats exposed to micronized BT-CNT.

1494 COMBINED EXPOSURE TO CARBON NANOTUBES AND BACTERIA ENHANCES PULMONARY INFLAMMATION AND INFECTIVITY.

A. R. Murray[1, 2], E. Kisin[1], J. P. Fabisiak[3], J. R. Roberts[1, 2], J. M. Antonini[1], C. Kommineni[1], J. Reynolds[1], A. Barchowsky[3], V. Castranova[1, 2], V. Kagan[3] and A. A. Shvedova[1, 2]. *[1]PPRB, NIOSH, Morgantown, WV, [2]Physiology and Pharmacology, West Virginia University, Morgantown, WV and [3]Environmental and Occupational Health and Pharmacology, University of Pittsburgh, Pittsburgh, PA.*

Carbon nanotubes (CNT) – with their applications in industry and medicine - may lead to new risks to human health. CNTs induce a robust pulmonary inflammation and oxidative stress in rodents. Realistic exposures to CNT may occur in conjunction with other pathogenic impacts (microbial infections) and trigger enhanced responses. We evaluated interactions between pharyngeal aspiration of single walled CNT (SWCNT) and bacterial pulmonary infection of C57BL/6 mice with Listeria monocytogenes (LM). Mice were first given SWCNT (0, 10 and 40 μg/mouse) and 3 days later exposed to LM (10^3 bacteria/mouse). Combined exposure to SWCNT/LM amplified lung inflammation and fibrosis. Despite this robust inflammatory response, SWCNT pre-exposure significantly decreased the pulmonary clearance of LM measured 3 - 7 days after microbial infection vs PBS/LM treated mice. Failure of SWCNT-exposed mice to clear LM led to a continued elevation in nearly all major chemokines and acute phase cytokines into the later course of infection. In SWCNT/LM exposed mice, BAL PMNs, AMs and lymphocytes, as well as LDH levels, were increased compared to mice exposed to SWCNT or LM alone. Increased levels of collagen deposition were found in the lung of mice exposed to SWCNT/LM vs SWCNT or LM alone. Combined exposure to SWCNT/LM caused persistent changes in breathing rate patterns indicative of detrimental decline in the lung function. In conclusion, enhanced acute inflammation and pulmonary injury with delayed pulmonary bacterial clearance after SWCNT exposure may lead to increased susceptibility to lung infection in exposed populations.

1495 COMPARATIVE PULMONARY TOXICITY STUDY OF 3 DIFFERENT PRIMARY-SIZED TIO2 PARTICLES IN RATS.

N. Kobayashi, M. Naya, S. Endo, K. Yamamoto and J. Nakanishi. *National Institute of Advanced Industrial Science and Technology, Tsukuba, Ibaraki, Japan.*

Lung toxicity of 3 different-sized, well-characterized, anatase TiO2 particles was assessed in rats. The average primary size of these particles was approximately 7, 20, and 200 nm, respectively. Groups of male Crl:CD (SD) rats were intratracheally instilled with 5 mg/kg of the TiO2 particles dispersed in disodium phosphate solution. Following the instillations, the bronchoalveolar lavage fluid (BALF) of the rats was examined for inflammatory markers, and the histopathology of the lung, liver, spleen, and cerebrum at post-instillation timepoints of 24 hours, 3 days, 1 week, and 4 weeks was also examined.

In all the groups, toxicological effects were observed only in the lung and not in the liver, spleen, or cerebrum. The pulmonary inflammatory responses were different among the groups. In the 7 nm-TiO2-instilled group, the BALF measurements indicated significant increase in total cell and neutrophil numbers and in LDH and IL-6 concentrations. Further, macrophage infiltration in the alveoli and interstitium, inflammatory-cell infiltration, and hypertrophy of the alveolar epithelium cells were observed in the histopathological evaluations. In the 20 nm-TiO2-instilled group, the inflammatory responses were almost the same as in the 7 nm-TiO2-instilled group. However, no significant increases in the IL-6 concentrations in the BALF, or macrophage infiltration in the interstitium were observed. In the 200 nm-TiO2-instilled group, no significant differences were observed for any of the inflammatory biomarkers in the BALF. Further, in the 200 nm-TiO2-instilled group, the levels of the macrophage infiltration in the alveoli and inflammatory-cell infiltration were much lower than those observed in the 7 nm- TiO2 or 20 nm-TiO2-instilled groups.

All the above-mentioned TiO2 particle types produced only transient pulmonary inflammatory effects, and these changes were recovered at 1 month after the instillations.

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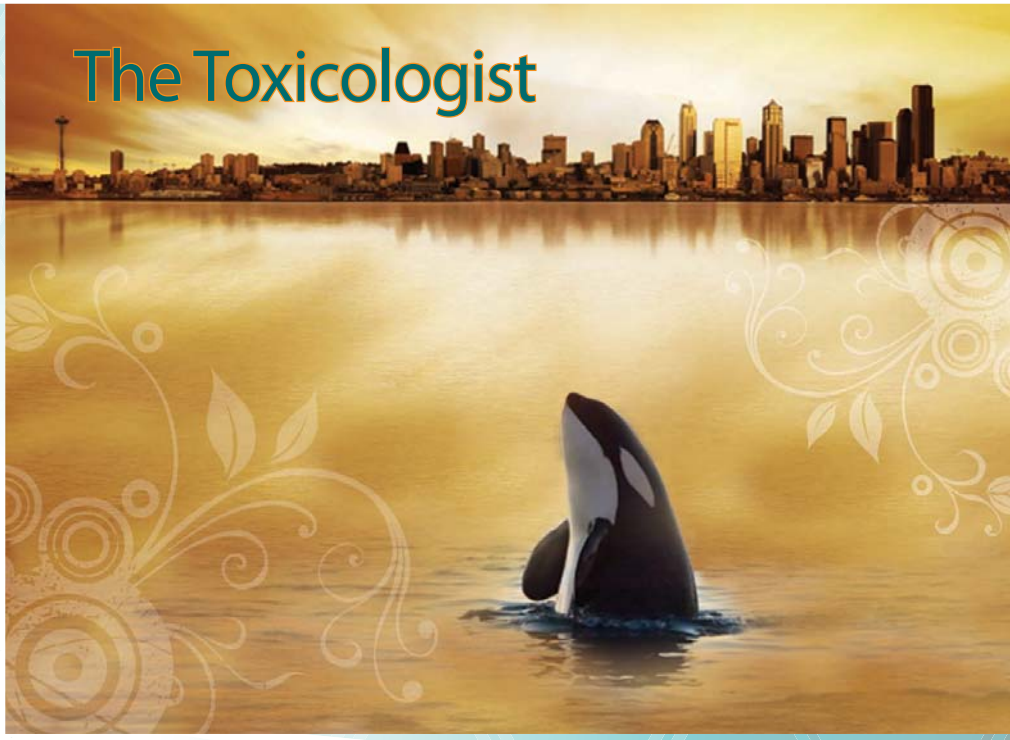

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