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.

SOT | Society of Toxicology

48th Annual Meeting and ToxExpo™

Baltimore, Maryland

March 15–19, 2009

Baltimore Convention Center

Photos courtesy of Baltimore Area Convention and Visitors Association

47th Annual Meeting and ToxExpo™

March 16–20, 2008

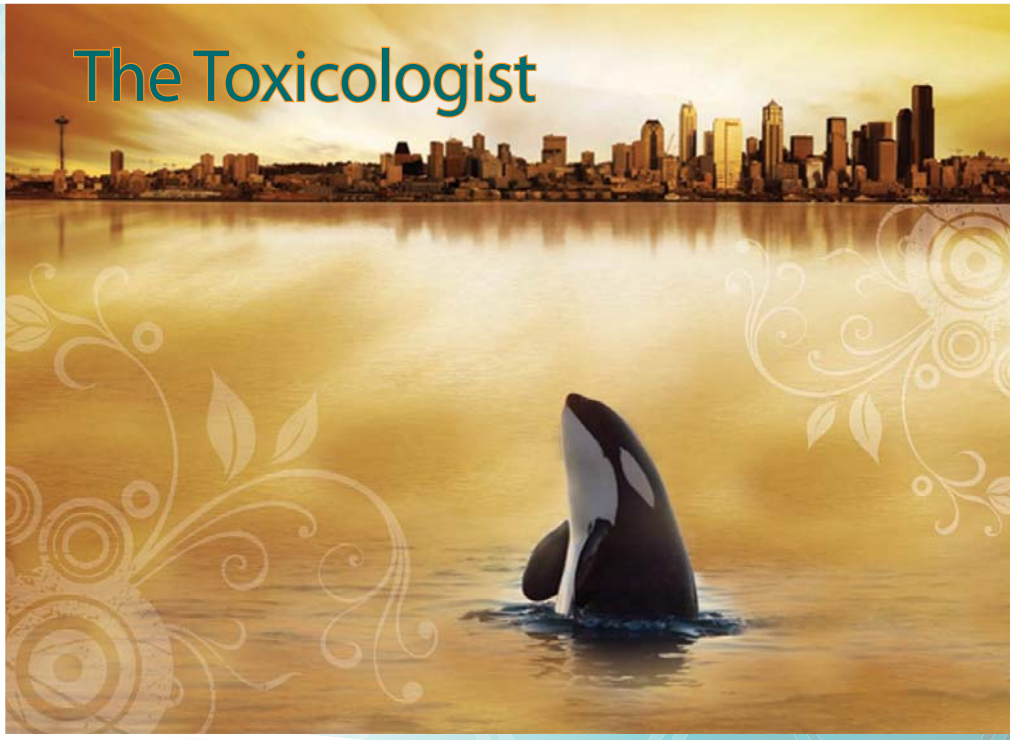
Society of Toxicology
1821 Michael Faraday Drive, Suite 300 • Reston, VA 20190
T: (703) 438-3115 • F: (703) 438-3113 • E-mail: sothq@toxicology.org
www.toxicology.org

SOT | Society of Toxicology

The Toxicologist

March 2008

The Toxicologist



47th Annual Meeting and ToxExpo™

Seattle, Washington

Supplement to Toxicological Sciences
An Official Journal of the Society of Toxicology

SOT | Society of Toxicology

www.toxsci.oxfordjournals.org

OXFORD UNIVERSITY PRESS ISSN 1096-6080 Volume 102, Number 1, March 2008