automated for long-term studies. Here we present the performance of this method and system. *This work was supported by the Health and Labour Sciences Research Grant, Japan.*

2224 Pulmonary Toxicity Associated with Different Zinc Nanoparticles after Intratracheal Instillation in Rats

X. Xin[1], M. Barger[1], K. Roach[2], G. Boyce[1], M. Duling[1], A. Stefaniak[1,2], S. Leonard[1,2], and J. Roberts[1,2]. *[1]NIOSH, Morgantown, WV; and [2]West Virginia University, Morgantown, WV.*

Zinc nanoparticles (Zn NPs) are used extensively in variety products including cosmetics, personal hygiene products, paints, food additives, and cancer therapeutic agents. As Zn NPs production increases, pulmonary toxicity related to occupational exposure becomes a concern. The goal of this study was to assess pulmonary toxicity of Zn NPs of different size, shape, solubility, and composition. In the first study, 2 doses (0.05 and 0.125 mg) of 6 particles were examined: fine-sized ZnO (FZnO), nano-sized elemental Zn (EZn), nano-sized ZnO (NZnO), ZnO nanowire (WZnO), and comparable nano-sized TiO_2 ($NTiO_2$) and TiO_2 nanowire ($WTiO_2$) as highly insoluble control materials. Male Sprague-Dawley rats were exposed by intratracheal instillation (II) to 0.05 or 0.125 mg of particles or vehicle control DM (dispersion medium) following a 5 min sonication on day 0. Body weights were recorded throughout the study. Parameters of lung injury and inflammation were measured in bronchoalveolar lavage (BAL) at 1 D, 7 D, 1 M and 3 M post-exposure. In a second study, rats were exposed to FZnO, EZn, and NZnO at the high dose in DM following a 30 sec sonication that resulted in less release of soluble Zn prior to II. The same time points and endpoints as in the first study were analyzed. In the first study, NZnO had the greatest specific surface area (SSA) and solubility prior to II. FZnO and NZnO agglomerates were smaller than the other samples, and consistent across studies. Study 1 showed the high dose of FZnO and NZnO caused decreased body weight on 1-3 D, which gradually increased by 1 M, followed by normal weight gain up to 3 M. All particles caused transient lung injury and inflammation at 1 D post-exposure except $NTiO_2$, with NZnO>FZnO>EZn>WZnO≥$WTiO_2$. At 7 D, lung injury and inflammation remained increased in the groups exposed to Zn NPs only. Inflammation persisted up to 3 M post-exposure to the greatest degree in the NZnO group followed by FZnO then EZn, while there was recovery in the groups exposed to TiO_2 and WZnO. Eosinophils were increased to the greatest degree in the NZnO > FZnO > EZn groups at 1 and 7 D. Study 2 showed a similar trend as the first study in changes of body weight, lung injury, and inflammation, although the difference between the NZnO and FZnO was not as pronounced. Material characterization studies suggested differences in toxicity may be associated with increased solubility as a function of composition (oxide vs elemental) and increased SSA.

2225 Bioactivity of Boron Nitride Nanotube Preparations That Differ in Purity *In Vitro* and *In Vivo*

J. Roberts[1,2], V. Kodali[1], X. Xin[1], M. Barger[1], K. Roach[2], A. Stefaniak[1,2], T. Eye[1], M. Wolfarth[1], S. Leonard[1], D. Porter[1,2], and A. Erdely[1,2]. *[1]NIOSH, Morgantown, WV; and [2]West Virginia University, Morgantown, WV.*

Boron nitride nanotubes (BNNTs), due to their wide band gap and thermal and chemical stability, are expected to be incorporated into a myriad of industrial applications. Currently, commercial production of BNNTs occurs through different processes including a pressurized vapor/high temperature process (PVTH) or an induction thermal plasma process (plasma), both resulting in 30-60 % residual compounds and impurities. In the current work, we evaluated the pulmonary and systemic toxicity arising due to acute exposure of BNNTs from the plasma process. Four BNNTs with a gradient of purity (from 50% to 90% tubes) were used to assess toxicity and evaluate bioactivity. Hexagonal boron nitride (less than 100 nm in diameter) was used as a reference material. All BNNTs tested were agglomerated bundles of few multi-walled tubes (~3 to 5 walls/tube). Electron microscopy (EM) confirmed a visible decrease in impurities and an increase in tubular structures across the gradient samples. *In vitro* and *in vivo* experiments were performed following sonication of BNNTs in dispersion media (DM). Preliminary EM sizing showed that the BNNTs dispersed in DM had a length of ~0.5 - 1.5 µm and a diameter of ~5 - 30 nm. Electron paramagnetic resonance measurements showed no change in surface hydroxyl radicals among the BNNTs with various purities. *In vitro*, the toxicity was evaluated in human monocyte cells (THP-1) wild-type and NLRP3-deficient cells at a concentration range of 0-100 µg/ml. At the high doses, there was a small but statistically significant increase in lactate dehydrogenase (LDH) released in the highest purity BNNT exposures. This increase in toxicity was attenuated in NLRP3-deficient cells. *In vivo* toxicity was evaluated in male C57BL/6 mice exposed by oropharyngeal aspiration to 4 or 40 µg of BNNT sample/mouse. Animals were euthanized 1 and 7 d post-exposure and lung lavage was performed to evaluate lung injury and inflammation. At day 1 there was a significant influx in neutrophils, a marker for lung inflammation, as well as an increase in LDH activity in particle-exposed groups. The response was highest in animals exposed to the high dose of the highest purity BNNT mixture. Inflammation and injury began to resolve by 7 d. The results indicate that BNNTs made by plasma process induce acute toxicity and inflammation only at high concentrations and ongoing studies will evaluate histopathological changes and clearance up to 3 mo post-exposure.

2226 Activities of Mitochondrial Enzymes in Heart and Brain of Rats Exposed to Titanium Dioxide Nanoparticles

O. Ademuyiwa[1], S. A. Kehinde[1], O. G. Adelesi[1], R. N. Ugbaja[1], E. O. Dare[1], D. O. Babayemi[1], and A. D. Wusu[2]. *[1]Federal University of Agriculture, Abeokuta, Nigeria; and [2]Lagos State University, Ojoo, Lagos, Nigeria.*

Titanium dioxide Nanoparticles (TiO_2 NPs) have found wide application in various products making exposure to this metal oxide nanoparticle inevitable. Information about toxic effects of TiO_2 NPs after oral exposure are extremely limited. Since mitochondrionopathies are being recognized as subtle and insidious biochemical events in the toxicity associated with various toxicants, this study determined the activities of mitochondrial enzymes (Malate dehydrogenase, MDH; Succinate dehydrogenase, SDH; Combined Complex I+III, CPI+III; Combined Complex II+III, CPII+III; Complex IV, CPIV) in rats exposed to TiO_2 NPs. Male albino rats were exposed orally to TiO_2 NPs (8-12nm) (50, 150 and 250mg/kg body weight) for 4, 8 and 12 weeks. Control rats (n=18) received distilled water for the same period. At the end of TiO_2 NPs exposure, brain and heart were removed from the rats and activities of mitochondrial enzymes determined. Enzymes in the two organs exhibited different patterns on exposure to TiO_2 NPs. While cardiac MDH was down-regulated throughout the study, brain MDH increased at 4 and 8 weeks of 50 and 12 weeks of 150mg/kg body weight doses respectively. In both organs, SDH activity was up-regulated at 4weeks. While the up-regulation in cardiac SDH was dose-dependent, brain SDH did not show any dose dependency. In contrast however, CPI+III in both organs was down-regulated, except at 4weeks of 150- and 12weeks of 50mg/kg body weight where increases ranging between 2 and 7 folds were observed. While cardiac CPII+III was decreased at 8 weeks of 150mg/kg body weight dose, the brain enzyme was up-regulated by 50 and 250mg/kg body weight doses of TiO_2 NPs. Unsystematic changes characterized the response of brain CPIV, whereas the cardiac enzyme was down-regulated, except at 8weeks of 50 and 250mg/kg body weight doses of TiO_2 NPs. Our findings indicate that TiO_2 NP-induced alterations in cardiac and brain mitochondrial energy metabolism might be important in pathologies associated with exposure to TiO_2 NPs.

2227 Crystalline Nanocellulose-Induced Lung Toxicity and Global Gene Expression Changes in the Rat

P. Joseph, T. Sager, T. Chen, W. McKinney, M. Orandle, J. Roberts, and C. Umbright. *NIOSH, Morgantown, WV.*

Crystalline nanocellulose (CNC) is an emerging nanomaterial with multiple commercial and industrial applications. Occupational exposure to CNC during the production and/or use of products containing the nanomaterial potentially resulting in adverse health effects among workers is possible. Therefore, there is an immediate need to determine the toxicity potential and mechanisms underlying CNC toxicity. Male Fischer rats were exposed by whole body inhalation exposure to air or CNC (20 mg/m^3, 6 hours/day, 14 days), and pulmonary toxicity and lung gene expression changes were determined one day following the last exposure. CNC particles were detected in the lung alveoli of the exposed rats. Compared with the air-only exposed controls, significant increases ($p<0.05$) in the incidence of bi-nucleated alveolar macrophages (AM), lactate dehydrogenase activity, pro-inflammatory cytokine levels, phagocyte oxidant production, and AM and neutrophil counts were detected in the bronchoalveolar lavage of the CNC exposed rats. Mild lung histological changes, such as the accumulation of AM and neutrophils, were observed in the CNC exposed rats. Global gene expression profiling by next generation sequencing identified 573 genes whose expressions were significantly different (fold change >1.5 and FDR $p<0.05$) in the lungs of the CNC exposed rats, compared with the controls. Bioinformatic analysis of the lung gene expression data identified significant enrichment of several biological functions and canonical pathways related to inflammation (cellular movement, immune cell trafficking, inflammatory diseases and response, respiratory disease, and free radical scavenging, complement system, acute phase response, leukocyte extravasation signaling, granulocyte and agranulocyte adhesion and diapedesis, IL-10 signaling, phagosome formation and matu-

ration, etc) and oxidative stress (NRF2-mediated oxidative stress response, production of nitric oxide and reactive oxygen species in macrophages, etc). In summary, the data demonstrated the induction of pulmonary toxicity and global gene expression changes in the lungs of the rats in response to inhalation exposure to CNC.

2228 The Impact of Neuroinflammatory Disease and Environmental Exposures on Gold Nanoparticle Accumulation in the Mouse Brain

C. Wong, B. Gelein, A. Kennell, G. Oberdörster, and A. Elder. *University of Rochester, Rochester, NY.*

Epidemiological studies correlate air pollution exposure with increased incidence of Alzheimer's Disease (AD). We postulate that translocation of inhaled ultrafine particles (UFP, <100nm) to secondary target organs, including the brain, is one mechanism by which airborne UFP can cause adverse effects. The biodistribution of nanoparticles, particularly to the brain, is still a question that needs to be thoroughly investigated. In young, healthy C57 mice (n=7) that were exposed for 4hrs via whole body inhalation (WBI) to gold nanoparticle aerosols (AuNP; count median diameter (CMD), 20nm, geometric standard deviation (GSD), 1.5), AuNP did not readily accumulate in the brain following an inhalation exposure. We hypothesize that inflammatory insults, such as environmental exposures or neuroinflammatory diseases, can change barrier permeability and ultimately alter NP biodistribution. Aged 3xTgAD mice - which mimic human AD-related pathologies and exhibit progressive neuroinflammation - and non-transgenic (NTg) mice (16 mo males, n=7-8) were exposed via WBI to AuNP aerosol for 4 hrs (CMD, 27 nm, GSD, 1.5). Tissues were harvested 24 hrs post-exposure and analyzed for Au using inductively-coupled plasma mass spectrometry. There was no significant difference of Au accumulation by genotype in the brain regions measured. Separate groups of aged 3xTgAD and NTg mice were also exposed to ambient UFP-enriched aerosols for 4hrs/day for 8 days (CMD, 88 nm, GSD, 1.5) as a purported air-blood barrier insult prior to AuNP aerosol exposure to characterize exposure-related changes in AuNP accumulation in the brain. UFP-enriched aerosol exposures did not induce acute lung inflammation nor significantly affect AuNP accumulation in the brain regions measured. However, when 3xTgAD and NTg mice (3, 19, and 23 mo males, n=7-11) were injected intravenously with colloidal AuNP (primary particle size 20 nm, 45-49 µg per mouse), there was a significant age x genotype interaction effect ($p=0.006$) found in the whole brain, as well as in areas of the brain that are particularly affected by AD, such as the hippocampus ($p<0.05$). This suggests that as the pathology progresses, the brain becomes more permeable to vascular accumulation of AuNPs, but that stronger insults are needed to see any differences in brain accumulation of inhaled AuNP. *Funding: R01ES020332, T32ES007026, P30ES001247.*

2229 Limited Neurotoxicity from Neonatal Inhalation Exposure to Ultrafine Carbon Particles or Diesel Particulate in C57Bl/6

K. Morris-Schaffer, A. Merrill, M. Sobolewski, and D. Cory-Slechta. *University of Rochester Medical Center, Rochester, NY.*

Epidemiological studies have shown exposure to anthropogenic fine particulate matter is associated with adverse neurodevelopmental outcomes in children. Complementary studies using rodent models have shown that developmental exposure to ambient nanoscale particulate matter can lead to sex-specific neurotoxicity and learning deficits. However it is still unclear about the direct sources and particulate matter constituents that contribute to these deleterious outcomes. To better evaluate potential particulate matter contributors to developmental neurotoxicity, two studies were conducted to assess the potential developmental effects of pure ultrafine carbon particles (UFCP: median aerodynamic diameter: <40 nm) and nanoscale diesel particles (median aerodynamic diameter: <100 nm) on brain pathology and behavior in C57Bl/6 mice. The UFCP aerosol was generated from a spark-discharge setup and the diesel particles were generated from ultrasonic nebulization of dissolved National Institute of Standards and Technology Standard Reference Material 1650b (SRM 1650b). Separate inhalation exposure of each material with neonatal mice occurred on postnatal days 4-7 and 10-13 for 4hr/day, 4 days/week at a mass concentration of 50 µg/m^3 for UFCP and 100 µg/m^3 for SRM 1650b. Assessments of central nervous system pathology 24 hours following exposure showed no gross inflammation or injury following pure ultrafine carbon exposure, while SRM 1650b did increase glial fibrillary acidic protein (GFAP) immunodensity in the corpus callosum and cortex, suggestive of inflammation. To assess learning deficits, behavior on a fixed-interval schedule of reinforcement, a paradigm that involves temporal learning and is historically effective at detecting the protracted effects of low-dose neurotoxicants such as lead, was utilized in exposed adult. No significant treatment-related learning differences were found in the adult mice. The lack of extended effect from the developmental particulate matter exposures, even at relatively high mass concentrations, suggests neither ultrafine elemental carbon nor diesel particle exposure alone are sufficient contributors to adverse developmental neurotoxicity. Further research on more reactive constituents of particulate matter including volatile organic species, reactive metals, and gases need to be done to better clarify specific toxic contributors.

2230 Activities of Glycolytic Enzymes in Rats Exposed to Titanium Dioxide (TiO_2) Nanoparticles (NPs)

S. Kehinde, O. Adelesi, R. Ugbaja, E. Dare, and O. Ademuyiwa. *Federal University of Agriculture, Abeokuta, Nigeria.*

Titanium dioxide Nanoparticles (TiO_2 NPs) have been applied widely in various products such as food, packaging, electronics, sunscreens, paints, drugs and cosmetics. Safety of TiO_2 NPs remains unclear because data on absorption, distribution, elimination or any adverse effects after oral exposure are extremely limited. Since disorders of energy metabolism are being recognized as incipient biochemical events in the toxicity associated with various toxicants, this study determined the activities of glycolytic enzymes in rats exposed to TiO_2 NPs. Male albino rats were exposed orally to TiO_2 NPs (8-12nm) (50, 150 and 250mg/kg body weight) for 4, 8 and 12 weeks. Control rats (n=18) received distilled water for the same period. At the end of TiO_2 NPs exposure, blood and liver were removed from the rats. Activities of glycolytic enzymes (Hexokinase [HK], Aldolase [ALD] and Lactate dehydrogenase [LDH]) were then determined. In the lymphocytes (with the exception of 50mg/kg body weight, 8 weeks and 150mg/kg body weight, 12 weeks), exposure to TiO_2 NPs up-regulated HK activity. Plasma and erythrocyte HK activities were also up-regulated except at 12 weeks of 150mg/kg body weight dose where 40 and 30% decreases were observed in the plasma and erythrocytes respectively. Hepatic HK followed the same pattern as that of the lymphocytes. In a similar vein, both hepatic and lymphocyte ALD followed the same pattern in their responses to TiO_2 NPs. Their activities were up-regulated except at 8 and 12 weeks of 50 and 150mg/kg body weight where slight decreases were observed. While plasma ALD also increased (except at 12 weeks of 150mg/kg body weight), erythrocyte ALD increased only at 4 and 8 weeks, but decreased throughout the 12 week exposure period. Compared to erythrocyte LDH that decreased on exposure to TiO_2 NPs, a common signature was observed for the lymphocyte, plasma and hepatic enzymes. LDH in these compartments was down-regulated at 8 and 12 weeks of 50 and 150mg/kg body weight doses of TiO_2 NPs, whereas at other doses and time intervals, LDH was up-regulated. Our findings indicate that while exposure to TiO_2 NPs inhibited erythrocyte glycolytic pathway at the level of ALD and LDH, it enhanced the flux through the plasma, lymphocyte and hepatic glycolytic pathways.

2231 Effects of Multi-Walled Carbon Nanotube Exposure on Brain Oxidative Stress and Inflammation in C57BL/6 Mice

T. L. Young[1], G. Herbert[1], S. Lucas[1], B. Sanchez[1], J. Begay[1], A. K. Ottens[2], A. Erdely[3], and M. Campen[1]. *[1]University of New Mexico, Albuquerque, NM; [2]Virginia Commonwealth University, Richmond, VA; and [3]NIOSH, Morgantown, WV.*

In vivo models demonstrate increased brain and lung inflammatory activation, disrupted blood-brain barrier (BBB) integrity, increased oxidative stress, serum profile changes, impaired endothelial function, and vasodilatory insufficiencies following multi-walled carbon nanotube (MWCNT) exposure. The mechanisms by which lung exposure to MWCNTs mediate neuroinflammation are explored in this study. We hypothesized that the MWCNT exposure induces serum peptide composition modifications that mediate pro-inflammatory and pro-oxidative stress changes in the brain, likely delivered via exosomes. To test this hypothesis, male wild-type C57BL/6 mice (6-8 weeks) exposed to vehicle (dispersion media), 3, 10 or 40 µg MWCNT via oropharyngeal aspiration were euthanized at various time points post exposure. Pulmonary inflammation was assessed via bronchoalveolar lavage fluid (BALF) cell and protein quantification and inflammatory cytokine/chemokine expression was determined by electrochemiluminescence. Serum bioactivity of whole and exosome-depleted fractions was assessed in mouse brain endothelial cells via serum cumulative inflammatory potential (SCIP) assay. Vasodilatory changes were determined via myography. Brain glutathione levels were measured using colorimetric assay to evaluate brain oxidative stress changes. Neuroinflammation and BBB changes were assessed via immunofluorescence staining. MWCNT exposure significantly increased macrophage and neutrophil infiltration in BALF. Cytokines (IL-1β, IL-4, IL-5, TNF-α and KC/GRO) were elevated in the BALF of MWCNT-exposed mice. Brain immunofluorescent

58TH ANNUAL MEETING & ToxExpo · MARCH 10–14, 2019

The Toxicologist

Supplement to *Toxicological Sciences*

OXFORD
UNIVERSITY PRESS

ISSN 1096-6080
Volume 168, Issue 1
March 2019

www.academic.oup.com/toxsci

The Official Journal of the Society of Toxicology

SOT | Society of Toxicology

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

www.toxicology.org

Publication Date: February 18, 2019