receive much attention for their potential usages in biosensing, bioimaging, and drug delivery. With the potential biomedical applications of CNDs, knowing and understanding their route of uptake into a cell is a critical piece of information that is still unanswered. In this study, we examined the possible route of uptake of CNDs within human microvascular endothelial cells (HMECs) by using several known transport inhibitors. The result showed that CNDs can uptake into HMECs, which utilize the intrinsic fluorescence of CNDs that has an emission signal at 460 nm upon excitation with a 360 nm laser. CNDs uptake was significantly affected by ebselen, which is a known inhibitor of mammalian H+, K+-ATPase, n-phenylanthranilic acid, a known chloride channel inhibitor, and chlorpromazine, a known suppressor of clathrin disassembly. Our data has suggested that CNDs might use different pathways to enter endothelial cells.

2156 ICONS: Integrated Testing Strategy for Mechanistically Assessing the Respiratory Toxicity of Functionalized MWCNT

O. H. Creutzenberg[1], D. Lison[2], S. Van Den Brule[2], F. Turcu[3], J. C. Bonner[4], and C. Ziemann[1]. *[1]Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany; [2]Louvain Centre for Toxicology and Applied Pharmacology, Louvain, Belgium; [3]Universitatea Babeş-Bolyai, Cluj, Romania; and [4]North Carolina State University, Raleigh, NC.*

Toxicological concerns are opposed to the promising technical properties of multiwalled carbon nanotubes (MWCNT). Meeting WHO fiber criteria MWCNT can be carcinogenic (rigid type) or non-carcinogenic (tangled type) depending on fiber length and diameter. The project ICONS focused on the comparison of core MWCNT vs. surface-modified MWCNT (both tangled type) regarding their fibrotic and genotoxic potential. Purification of core MWCNT (chemically or thermally) and surface functionalization (-COOH or -NH2) of the industrially relevant Nanocyl NC7000 were varied. At Fraunhofer ITEM, the eight resulting MWCNT (pristine, milled, purified, and functionalized) were tested for sterility and endotoxin contamination. For *in vitro* use, they were dispersed using an ultrasound-based protocol, and characterized by light and scanning electron microscopy. Subsequent *in vitro* (geno)toxicity testing with MRC-5 primary human lung fibroblasts revealed differential inhibition of proliferation (RICC, mitotic index) and induction of membrane damage, DNA-strand breaks and micronuclei. Using primary human mesothelial LP9 cells, a variable number of differentially expressed genes was noted. Based on these *in vitro* data and the *in vivo* data, generated by LTAP and NCSU, the COOH-functionalized chemically purified (NC3151) and thermally purified (NC-PlacylCOOH) samples were selected for a 4-wk inhalation study in rats (design based on OECD TG 412; 0.2, 1 and 5 mg/m^3), including a 4-wk recovery (validation test). As a reference group, the thermally purified NX7100 sample (non-functionalised core) was included with 5 mg/m^3 only. Pre-trials demonstrated feasibility of generating respirable MWCNT aerosols by dry dispersion with pressurized air, supported by a jet mill. The differential cell count analysis, the levels of lactic dehydrogenase, beta-glucuronidase and total protein in bronchoalveolar lavage fluid and the histopathological examination resulted in the following ranking: NC3151 < NX-7100 (core) = NC-PlacylCOOH, indicating that the purification method seems to be important. *This ERA-NET SIINN project was funded by the German BMBF (FKZ: 03XP0063).*

2157 Investigating Barrier Capacity of Human Placenta to Foodborne TiO_2 Nanoparticles (E171) Using an *Ex Vivo* Perfusion Model and Ti Quantification in the Placenta and Meconium

A. Guillard[1], E. Gaultier[1], C. Cartier[1], L. Devoille[2], J. Noireaux[2], L. Chevalier[3], F. Grandin[1], C. Coméra[1], M. Morin[4], C. Vayssière[4], N. Feltin[2], N. Bekhti[5], K. Adel-Patient[5], K. Chardon[6], V. Bach[6], P. Fisicaro[2], N. Picard-Hagen[1], and E. Houdeau[1]. *[1]Toxalim INRA, Toulouse, France; [2]LNE, Paris, France; [3]GPM CNRS, Rouen, France; [4]Centre Hospitalier Universitaire de Toulouse, Toulouse, France; [5]SPI CEA-INRA, Saclay, France; and [6]PERITOX, Université de Picardie Jules Verne, Amiens, France.* Sponsor: R. Barouki

Food-grade titanium dioxide (TiO_2, white pigment E171 in EU) contains up to 55% (number-based) of nanoparticles (NPs). Despite low intestinal absorption, human dietary exposure is chronic (0.2-10 mg/kg/day) and TiO_2-NPs pass to the bloodstream and accumulate in systemic organs. In rodents, perinatal exposure to TiO_2-NP models showed transplacental transfer with health effects in offspring. The current study aims at evaluating whether the NP fraction of E171 may cross the human placenta. Term placentae and meconium were collected after delivery with mother consents. Basal titanium (Ti) levels were assessed by inductively coupled plasma-mass spectrometry (ICP-MS). Crystal TiO_2 forms were assessed on placenta tissue sections and fetal exudate by energy dispersive X-ray analysis (EDX) coupled with transmission and scanning electron microscopy (TEM/SEM-EDX), respectively. Placentae were perfused in a double open circuit for 30min of equilibrium with Earle's medium (EM), followed by 1h of EM alone (controls, n=2) or supplemented with E171 (15µg/ml, n=7). Passive antipyrine transfer rate served as viability marker. Passage of laser-reflective particles was evaluated by confocal microscopy on fetal exudate every 5min, and particle nature and size analyzed by SEM-EDX and ImageJ software. Basal Ti levels were 0.10±0.13 mg/kg in 92% of placentae (n=23/25), and 0.19±0.13 mg/kg in 36% of meconium (n=4/11). Anatase and rutile TiO_2 particles were commonly found in placentae. Laser-reflecting particles were detected in fetal exudate 10min after E171 addition in the maternal side. SEM-EDX imaging showed TiO_2 particles of diameters <200nm in the fetal side, 83% of them were NPs. In conclusion, circulating TiO_2 accumulates in the human placenta with Ti recovered in the meconium, demonstrating materno-fetal passage. *Ex vivo*, a placental transfer of E171 (TiO_2) particles mostly concerned NP fraction. These data emphasize the need of risk assessment in pregnant women of chronic exposure to TiO_2-NPs of dietary origin.

2158 DNA Damage Caused by Nickel Nanoparticle Exposure in Lung Epithelial Cells

Y. Mo, Y. Zhang, and Q. Zhang. *University of Louisville, Louisville, KY.*

Nickel and nickel compounds are highly carcinogenic and nickel nanoparticles (Nano-Ni) are becoming increasingly used in industry (e.g. magnetic tape, pastes, catalytic materials, microfilters, etc). More interestingly, due to unique chemical and physical properties of nanostructure, nickel alloy nanomaterials have received special interest in biomedical applications. Our previous studies showed that exposure to Nano-Ni caused severe and persistent lung inflammation, which were strongly associated with pulmonary toxicity. In addition, exposure to Nano-Ni caused HIF-1α nuclear accumulation. The overall hypothesis of this study is that Nano-Ni may exert genotoxic effects via alternation of cell homeostasis through a mechanism mediated by activation of ataxia-telangiectasia mutated (ATM) and HIF-1α accumulation. We compared the ability of non-toxic doses of Nano-Ni and Nano-TiO_2 to cause DNA damage and explored the possible mechanisms. Our results showed that exposure of normal human bronchial epithelial cells BEAS-2B to Nano-Ni caused a dose- and time-response increase in the expression of phosphorylated histone H2AX (γ-H2AX), Rad51 and phosphorylated p53, indicating DNA damage. However, exposure to Nano-TiO_2 did not cause those effects. To investigate the potential pathways involved in the Nano-Ni-induced DNA damage, we determined the phosphorylation of ATM and found that phosphorylation of ATM was increased when BEAS-2B cells were exposed to Nano-Ni. Furthermore, pre-treatment with KU55933, a specific inhibitor of ATM, suppressed Nano-Ni-induced p53 phosphorylation and Rad51 expression. Our results also showed that exposure of BEAS-2B cells to Nano-Ni caused HIF-1α accumulation, up-regulation of miR-210, and down-regulation of Rad52. Rad52 is a key factor in homologous recombination repair. Our *in vivo* studies also showed that exposure of mice to Nano-Ni caused increased expression of phosphorylated γ-H2AX and Rad51, and enhanced phosphorylation of p53 in the lungs. Thus, Nano-Ni-induced DNA damage may be induced by Nano-Ni-induced ATM activation and HIF-1α accumulation. These findings have important implications for understanding the potential health effects of nanoparticle exposure. *This work was partly supported by ES023693, ES028911, and HL147856 from NIH, and Kentucky Lung Cancer Research Program to Dr. Qunwei Zhang.*

2159 Modeling the Influence of Carbon Nanotube and Nanofiber Physicochemical Properties on Key Molecular Initiating Events and Functional Endpoints Using Epithelial, Macrophage, and Fibroblast Cell Models

V. K. Kodali[1], F. Kelly[1], J. Hubczak[1], K. Siegrist[2], A. K. Bauer[2], L. M. Sargent[1], M. Shoeb[1], T. A. Stueckle[1], T. Eye[1], A. Stefaniak[1], M. M. Dahm[1], M. Schubauer-Berigan[1], T. Boots[1], N. Yanamala[1], and A. D. Erdely[1]. *[1]NIOSH, Morgantown, WV; and [2]University of Colorado Anschutz Medical Campus, Aurora, CO.*

There is a significant interest in using *in vitro* systems to evaluate toxicity of ever-increasing nanomaterial variants and other toxicants. We used 7 multiwalled carbon nanotubes and 2 carbon nanofibers (CNT/F) from U.S facilities to evaluate predictability, sensitivity, and the relationship of physicochemical characteristics to key molecular initiating events and functional responses following exposure. Additionally, the predictability of *in vitro* model systems to *in vivo* outcomes from a complementary study was determined. The CNT/F represent a good model as they are known to induce cytotoxicity, inflammation, pathology, and genotoxicity. The particulates had a wide distribution in

length (0.1-50 µm), diameter (6-397 nm), dustiness (0.2-4.9 %), metal contaminants (0.3-6.2 %), surface area (18–238 m2/g), and density (0.007–0.22 g/cm3). Endotoxin and PAH levels were below detection limit and zeta potential were similar for all materials. Genotoxicity was evaluated in human lung epithelial cell line, BEAS-2B, at 0-24 µg/ml. Acute toxicity, inflammation, inflammasome signaling, and phagocytic activity were evaluated in the differentiated human monocyte cell line, THP-1, at 0-60 µg/ml. Collagen production, TGFβ levels, and αSMA signaling were evaluated in primary human lung fibroblast cells at 0-9.6 µg/ml. Unsupervised approaches were initially used to identify classes of materials with similar outcomes followed by supervised learning approaches to identify specific physicochemical characteristics driving toxicity responses. It was clear certain physicochemical characteristics were the primary drivers of specific outcomes. Often, a multifactorial approach, meaning a combination of physicochemical characteristics, best described a particular outcome. Analysis of complementary endpoints in a concurrent *in vivo* study indicated some *in vitro* tests shared similar predictability suggesting some utility for predictive *in vitro* toxicity evaluation. These included specific measures of inflammation and pathological outcomes. The general conclusions of the analysis suggest that the class of materials, carbon nanotubes and nanofibers, can be subdivided based on specific endpoints, some aspects of *in vitro* outcomes predict *in vivo* toxicity, and the methodological approach can possibly be adapted beyond the finite scope of this study.

PS 2160 The Role of Macrophage Surface Receptors on the Uptake and Binding of Protein-Coated MWNTs

M. Huynh, C. Mikoryak, P. Pantano, and R. Draper. *University of Texas at Dallas, Richardson, TX.*

Multi-walled carbon nanotubes (MWNTs) pose a human respiratory hazard because they can cause pulmonary fibrosis, which may lead to mesothelioma. A key event in the fibrotic pathway is the interaction of MWNTs with macrophages that may result in chronic inflammatory responses, but the mechanisms are not clear. An important question is whether macrophages have MWNT receptors that might initiate or modulate signals involved in inflammation. A complicating factor is that *in vivo* a protein corona, derived from serum proteins such as bovine serum albumin (BSA), may influence the interaction of MWNTs with cells. The interaction of BSA-coated carboxylated MWNTs (cMWNTs) and pristine (pMWNTs) with mouse RAW 264.7 macrophage cells was studied using a gel electrophoresis assay to quantify cell-associated MWNTs (Wang et al., *Anal. Chem.* 2009, 81, 8, 2944-2952). Both MWNT types accumulated as a function of time and concentration. To assess the potential role of cell surface receptors on macrophages, the binding of BSA-coated cMWNTs and pMWNTs to RAW 264.7 cells at 4°C in medium without serum was measured. At low temperature phagocytosis is inhibited, so that only binding of MWNTs on the cell surface is measured. Further, the absence of serum eliminates complications in interpreting the data that could arise due to the interaction of other serum proteins with the MWNTs to form an undefined protein corona. These studies directly demonstrated binding of both MWNT types to the cell surface that was a saturable function of MWNT concentration, supporting the idea that receptors bind BSA-coated MWNTs. The effect of BSA on the binding of BSA-coated MWNTs to the cells at 4°C showed that BSA reduced binding by 50%. Previous work suggests that scavenger receptors on macrophages bind cMWNTs (Wang et. al., Nanotoxicology, 2018, 12, 7, 677-698, DOI: 10.1080/17435390.2018.1472309). We found here that dextran sulfate, a known antagonist of Class A scavenger receptors, inhibited the binding of BSA-coated MWNTs to RAW 264.7 cells by a maximum of 50%. This suggests that on these macrophages there might be dextran sulfate sensitive and insensitive receptors.

PS 2161 Toxic Effects of Molybdenum Trioxide Nanoparticles (MoO_3 NPs) on Rat Pleural Mesothelial Cells (RPMCs) and Lavaged Cells from the Lungs of Golden Syrian Hamsters

E. A. Huber, and J. M. Cerreta. *St. John's University, Queens, NY.*

MoO_3 NPs are used in industrial, agricultural and biomedical applications due to their physical characteristics. The risk of exposure and possible adverse health effects to humans increases during the manufacturing and handling of such materials. The purpose of this study was to investigate the toxicity of MoO_3 NPs in cultured cells and in the cells isolated from bronchoalveolar lavage fluid (BALF) from Golden Syrian hamsters. Cultures of RPMCs (CCL-216) were exposed to MoO_3 NPs (100, 200, 300, 400, 500, 600, 700 or 800µg/mL) for 24 hours and compared to a vehicle control. Cytotoxicity was assessed by MTT and LDH assays. A concentration of 400µg/mL MoO_3 NPs was further evaluated (~LC_{50}). Capsase 1 and 3 protein levels were also measured in both groups. In addition, hamsters were exposed via inhalation and divided into four groups: 1) no exposure, 2) exposure to aerosolized water for 4h/day for 8 days, 3) exposure to 5mg/m^3 MoO_3 NPs for 4h/day for 8 days (*5-NP*) and 4) a group given a recovery period of one week (*5-REC*) following a *5-NP* exposure. Cultures of RPMCs treated with MoO_3 NPs had increased levels of: LDH (178%), caspase 3 (14%) and caspase 1 (29%) as compared to a control. BALF from the *5-NP* group had increased: total protein levels (62%), total cell counts (58%), neutrophils (870%) and multinucleated macrophages (465%) as compared to controls. BALF from the *5-REC* group had displayed parameters similar to controls, but had an increase of lymphocytes (938%) compared with controls. Tissue sections from the lungs of MoO_3 NPs treated groups of hamsters had membrane blebbing and hyperplasia of the airway epithelia with areas of cell proliferation at the periphery of the lung. Hamsters of the *5-NP* group had increased TUNEL positive cells in airway epithelia. Results from this study indicate cytotoxicity in RPMCs treated with 400µg/mL MoO_3 NPs may be mediated by both oncosis and apoptotic pathways. Animals exposed to 5mg/m^3 MoO_3 NPs had an initial acute inflammatory reaction as indicated by an alteration of BALF cells and protein levels that resolved to a chronic reaction with increased lymphocytes and hyperplasia in the airways. Cellular membrane blebbing in airway epithelia and an increase in TUNEL positive cells in hamsters exposed in the *5-NP* group support apoptosis as a possible pathway of injury.

PS 2162 *In Vivo* Lung Toxicity Associated with Boron Nitride Nanotubes with Different Purities

X. Xin[1], M. Barger[1], K. A. Roach[1], L. Bowers[1], V. Kodali[1], M. B. Jakubinek[2], K. Kim[2], S. Dénommée[2], M. G. Wolfarth[1], S. S. Leonard[1], D. W. Porter[1], A. D. Erdely[1], and J. R. Roberts[1]. *[1]NIOSH, Morgantown, WV; and [2]NRC Canada, Ottawa, ON, Canada.*

Boron nitride nanotubes (BNNTs), a newly emerging nanomaterial with enhanced physicochemical properties, are increasingly incorporated into industrially relevant applications. Currently, commercial production of BNNTs is synthesized with 30-60 % residual compounds and impurities. The goal of this study was to assess the lung toxicity associated with *in vivo* exposure to BNNTs with various purities. Three BNNT samples with a gradient of purity were provided by the National Research Council, Canada, to assess lung toxicity *in vivo*: a low purity as-produced sample with ~50% BNNT (BNLP), an intermediate purity sample (BNMP), and a highly purified sample with >90% BNNT (BNHP). Hexagonal boron nitride (h-BN, <100 nm in diameter) was used as a control material for one of the by-products during synthesis affecting the purity of the BNNT material. All BNNT samples tested were shown to be agglomerated bundles of BNNTs (~3 to 5 walls/tube) with boron and h-BN as the primary impurities. Sample purity was confirmed by electron microscopy (EM). The BNNT samples prepared in dispersion medium (DM) were 0.5-1.5 µm in length and 5-30 nm in diameter. Male C57BL/6 mice were exposed by oropharyngeal aspiration to 4 or 40 µg of sample/mouse dispersed in DM or DM alone on day 0. Animals were euthanized at 4 h, 1 d, 7 d, 1 m, and 3 m post-exposure and lung lavage was performed to evaluate lung injury and inflammation. At 4 h post-exposure, lactate dehydrogenase (LDH) activity and neutrophils influx, indicators of lung injury and inflammation, were significantly increased by high dose of BNMP and BNHP. At 1 d and 7 d post-exposure, the effects were greatest in the high dose of all three tested BNNT samples, with BNHP>BNMP>BNLP, and persisted in the BNHP group up to 1 m post-exposure. Irritant response, indicated by eosinophils increase, was observed in the high dose of BNNT groups at 1 d and 7 d post-exposure, with BNHP>BNMP>BNLP. Lung lymphocytes continued to increase in the BNHP group up to 1 m post-exposure. The results indicated that the tested BNNT samples induced acute toxicity and inflammation only at high concentration and the effects were more pronounced with increasing purity. The reference material used to represent one of the by-products of synthesis, h-BN, did not show significant lung toxicity, suggesting lung effects where present may be due primarily to BNNTs.

PS 2163 Analysis of Nanoparticle Uptake by Disease Vector of Economic Importance

T. Ameh[1], K. Zarzosa[2], T. Varzeas[1], B. Sharp[1], M. Gibb[1], C. Sayes[1], and E. Braswell[2]. *[1]Baylor University, Waco, TX; and [2]USDA Animal and Plant Health Inspection Service, Edinburg, TX.*

The spread of vector-borne pathogens by arthropod hosts continue to cause human, animal and plant diseases of public health and economic importance. Using the integrated vector management approach, this study utilizes nanoparticles as potential targeted anti-pathogenic agents for the control of citrus greening disease. *Candidatus Liberibacter asiaticus*, the bacterial pathogen of citrus greening disease is spread by the Asian citrus psyllid (*Diaphorina citri Kuwayama*). Symptoms of the disease include blotchy mottle, yellow shoots, and improperly developed fruits. The significant decrease in edible

SOT 2020

59th Annual Meeting & ToxExpo

Anaheim, California • March 15–19

The Toxicologist

Supplement to *Toxicological Sciences*

Toxicological Sciences

ISSN 1096-6080
Volume 174, Issue 1
March 2020

The Official Journal of the Society of Toxicology

OXFORD UNIVERSITY PRESS

SOT | Society of Toxicology

www.academic.oup.com/toxsci

Publication Date: February 21, 2020

Preface

This issue is devoted to the abstracts of the presentations for the Continuing Education courses and Scientific Sessions of the 59th Annual Meeting of the Society of Toxicology, held at the Anaheim Convention Center, Anaheim, California, March 15–19, 2020.

An alphabetical Author Index, cross-referencing the corresponding abstract number(s), begins on page 542.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 580.

The abstracts are reproduced as accepted by the Scientific Program Committee of the Society of Toxicology and appear in numerical sequence. If a number is missing in the numerical sequence, the abstract assigned to the missing number was withdrawn by the author(s). Author names that are underlined in the author block indicate that the author is a member of the Society of Toxicology. For example, J. Smith. SOT members may sponsor abstracts that do not include an author with SOT membership. Authors who are members of designated organizations could serve as the sponsor of the abstract if an SOT member was not a co-author; these types of sponsorships are displayed with an organization name after the sponsor name (e.g., Sponsor: A. Smith, EUROTOX).

Scientific Session Types:

- CE Continuing Education Courses
- EC Education-Career Development Sessions
- IS Informational Sessions
- PL Platform Sessions
- PS Poster Sessions
- RI Regional Interest Sessions
- R Roundtable Sessions
- S Symposium Sessions
- W Workshop Sessions

The 2020 SOT Event App and Online Planner

The Event App is available via the SOT Annual Meeting website and app marketplaces. The Event App, alongside the Online Planner available on the SOT Annual Meeting website, enables attendees to engage with organizers, exhibitors, and each other and to manage their time and maximize their experience during the Annual Meeting. ePosters also can be accessed electronically via the Event App until May 15, 2020.

To cite a 2020 SOT Annual Meeting abstract, please format as follows: *The Toxicologist*, Supplement to *Toxicological Sciences*, 174 (1), abstract #__, 2020, Title, First Author.

Society of Toxicology
11190 Sunrise Valley Drive, Suite 300, Reston, VA 20191

www.toxicology.org