

responses. We found that the biological response(s) elicited by fullerenes on interaction with lung cells may depend upon their ability to perturb cell cycle checkpoints potentially inducing senescence. Further elucidation of the underlying molecular mechanisms involved in this senescence response indicated the involvement of GADD45a, p16, p21 and p53a, a response characteristic of cells undergoing senescence. Finally, we correlated the physicochemical properties of engineered fullerenes with the observed biological responses to obtain a better understanding of property-dependent bioactivity of fullerenes.

PS 448 An In Vitro Assay Detects Enhancement of Mouse T Cell Sensitization to Ovalbumin by Carbon Nanoparticles.

D. E. Lefebvre¹, B. Pearce¹, E. Chomyshyn¹, N. Ross¹, S. Halappanavar², A. F. Tayabali², I. Curran¹ and G. S. Bondy¹. ¹Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch, Health Canada, Ottawa, ON, Canada; ²Environmental Health Science and Research Bureau, Environmental and Radiation Health Sciences Directorate, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, ON, Canada.

RATIONALE AND SCOPE: Previous studies suggested that some nanomaterials can promote allergic sensitization. At present there are no in vitro tools to study this risk. The hypothesis was that an in vitro screening assay could be developed to assess the adjuvanticity of agglomerated carbon black nanoparticles (CBNP). **EXPERIMENTAL PROCEDURES:** DO11.10 transgenic mice have a T cell receptor which recognizes the ovalbumin (OVA) protein from chicken egg. Splenic leukocytes from these mice were cultured with 0, 0.012, 0.12, 1.2 or 12 µg/mL CBNP, OVA, or OVA with CBNP. T cell mitosis rate was quantified by flow cytometry on day 3 post-exposure. T helper (Th1/Th2) cytokine production was measured by qPCR and ELISA. **RESULTS:** Printex 90 and Aldrich carbon CBNP products were characterized. These powders consisted of micron-sized agglomerates made up of 22 nm and 39 nm diameter CBNP base particles, respectively. Following sonication in saline RP-10 solution, the fraction of agglomerates smaller than 220 nm was purified by filtration for cell exposure. These particles did not induce T cell mitosis, and they did not modify this parameter during the response to OVA. These CBNP alone did not induce Th1/Th2 cytokine expression. However, OVA in combination with 12 µg/mL of either Printex 90 or Aldrich carbon significantly increased the allergy-related Th2 cytokines IL-4, IL-10 and IL-13 compared with OVA alone (p<0.05; n=3-5/group). This was concurrent with a decrease in the Th1 transcription factor Stat4. Lower CBNP doses had no effect. **CONCLUSIONS:** An in vitro immunotoxicology tool was developed. At the highest dose, carbon nanoparticles enhanced allergy pathways in mouse immune cells responding to ovalbumin. This assay will be used to further characterize nanomaterials for risk assessment purposes.

PS 449 Role of Transforming Growth Factor-β1 Pathway in Carbon Nanotube Stimulated Collagen Production in Human Lung Cells.

A. Mishra^{1,2}, T. A. Stueckle¹, R. Derk¹, V. Castranova^{1,2}, Y. Rojanasakul², J. Yuan³ and L. Wang^{1,2}. ¹Pathology and Physiology Research Branch, Health Effects Laboratory Division, NIOSH, Morgantown, WV; ²School of Pharmacy, West Virginia University, Morgantown, WV; ³School of Public Health, Hebei United University, Tangshan, China.

Accumulated studies have shown that carbon nanotubes (CNT) induce rapid and progressive lung fibrosis in animal models but the mechanisms are not clear. Following CNT exposure transforming growth factor β (TGF-β), a pro-fibrogenic mediator, was induced both in vivo and in vitro models and was correlated with in vivo fibrosis and in vitro collagen induction. To understand the signaling mechanism of this fibrogenic response, we investigated the contribution of TGF-β signaling in CNT-induced collagen production, a hall mark of fibrosis, using cultured human lung cells and determined the role of TGF-β receptor-Smad (TGF-βR1-Smad) signaling as a potential mechanism for CNT-induced fibrosis. Human lung epithelial (BEAS2B) cells and fibroblast (CRL1490) cells were exposed to doses relevant to in vivo exposure (0.02-0.6 µg/cm² in vitro ~ 10-80 µg/mouse lung) of well characterized and dispersed multi-walled CNT (MWCNT), single walled CNT (SWCNT) and ultrafine carbon black (UFCB). Protein expression was measured by immunofluorescence, western blotting and ELISA. Present results indicate: 1) CNT exposure caused induction of TGF-β1 production in lung epithelial cells; 2) TGF-β, TGF-βR1, p-Smad-2, and collagen type I were overexpressed in CNT-exposed fibroblast cells; 3) collagen I stimulating effects of MWCNT were partially blocked in TGF-βR1 and Smad-2 knockdown fibroblast cells. In conclusion, CNT stimulate lung fibroblasts to induce collagen I in vitro through activation of the TGF-β R1-Smad Signaling pathway.

PS 450 Factors Associated with the Releasability of Carbon Nanotubes (CNTs) from Nanocomposites in Potential Consumer or Industrial Applications.

M. Kovoich, R. Avanasì and A. K. Madl. *ChemRisk, Aliso Viejo, CA.*

Engineered nanomaterials offer innovative advancements for a wide range of industrial and consumer product technologies which promise to have global economic impact. Engineered nanomaterials in composites (nanocomposites) are currently being used in applications ranging from basic consumer goods to critical national defense technologies, with carbon nanotubes (CNTs) being popular for nanocomposites due to their enhanced mechanical, thermal, and electrical properties. With comparisons of CNTs to other high aspect ratio fibers, some concerns have been raised regarding the potential implications for exposure and health risk of nanocomposites containing CNTs. We hypothesized that the physical and chemical interactions between CNTs and the composite matrix, as well as settings in which nanocomposites are handled will influence the release of these nanomaterials. We analyzed available data on the release of CNTs from different composites as a result of various stressors. Although no release was detected under UV weathering conditions, CNT surface aggregation was detected in thermoplastic and epoxy composites compared with cementitious material. Matrix type, nanomaterial dispersion within the matrix, and chemical bonding were critical determinants for releasability. Mechanical stress tests such as cutting, grinding, sanding, and abrasion showed both positive and negative releasability results. Taken together, data indicate that physical, chemical, and environmental factors can affect the release of CNTs from nanocomposites including the location of the CNTs within the matrix, the chemical and physical bonding between the CNTs and the matrix, as well as the physical stress applied to the matrix. Analytical methods distinguishing release of CNTs versus matrix nanoparticles are critical to characterizing nanomaterial exposure. Understanding the factors that play a role in the release of CNTs will aid in technological development and safe handling of nanocomposites while minimizing any potential health risks.

PS 451 In Vitro Endothelial Exposure to Carbon Nanotubes Produce Reactive Oxygen Species.

Y. Rodriguez Yanez¹, I. Poblete-Naredo¹, B. Chavez-Munguia², B. Cisneros³ and A. Albore¹. ¹Toxicology, Cinvestav, Mexico City, Mexico; ²Infectomics and Molecular Pathology, Cinvestav, Mexico City, Mexico; ³Genetic and Molecular Biology, Cinvestav, Mexico City, Mexico.

Recent studies are focused to carbon nanotubes (CNT) effects on blood coagulation, and have demonstrated that CNT are able to induce platelet aggregation and vascular thrombosis. However, there is little information on CNT effects on fibrinolysis. Therefore, we investigated the role of CNT on fibrinolysis and their contribution to elicit a prothrombotic process in vascular endothelium and the reactive oxygen species (ROS) participation. In the present study we examined the CNT oxidative potential by ROS production and the induction of fibrinolysis-related gene expression in human umbilical vein endothelial cells (HUVEC) isolated from the vein of the umbilical cord. Primary HUVEC cultures were exposed to single-walled carbon nanotubes (SWCNT) at 5, 25 and 50 µg/ml during 24 h, and oxidation potential (free-cell dithiotreitol oxidation assay), cytotoxicity (propidium iodide stain) and cell morphology (transmission electron microscopy, TEM) were assessed. SWCNT exposure resulted in concentration-dependent changes: a) oxidation potential increases that suggest a ROS increase and, b) viability decreases. Additionally, morphological changes in mitochondria, chromatin and nucleus were observed by TEM. It is expected that the oxidative stress caused by ROS may affect the transcription of the fibrinolysis related genes, activators: tissue- and urokinase-activator, tissue kallikrein, [tPA, uPA, KLK1]); and inhibitors: plasminogen activator inhibitor type 1 and kallistatin [serpine1, serpine4]), altering the physiological fibrinolysis pathway in the vascular endothelium (Supported by grants SSA/IMSS/ISSSTE/CONACYT grant 162391 and ICyTDF51/2012, YRY received a Conacyt scholarship 203482).

PS 452 Transport of Inhaled MWCNT to the Pleura, Respiratory Muscles and Systemic Organs.

R. R. Mercer, A. F. Hubbs, J. F. Scabilloni, L. Wang, L. A. Battelli, V. Castranova and D. W. Porter. *PPRB, NIOSH, Morgantown, WV.*

Inhalation exposure studies of mice were conducted to determine if multi-walled carbon nanotubes (MWCNT) distribute to the parietal pleura, respiratory musculature and systemic organs. Male C57BL/6J mice were exposed in a whole-body inhalation system to a 5 mg/m³ MWCNT aerosol for 5 hours/day for 12 days (4 times/week for 3 weeks). At 1 day and 48 weeks after the 12 day exposure period,

mice were anesthetized and lungs and systemic tissues were preserved by whole body vascular perfusion of paraformaldehyde while inflated with air. A separate, clean-air control group was studied. Sirius Red stained sections from lung, diaphragm, chest wall, heart, kidney and liver were analyzed. Enhanced darkfield microscopy and morphometric methods were used to detect and count MWCNT in tissue sections. Counts in tissue sections were expressed as number of MWCNT per cm² of tissue (mean±SE, N=8 mice per group). Although agglomerates account for approximately 60% of lung burden, only singlet MWCNT were observed in diaphragm, chest wall and systemic tissues. At one day post exposure, the average length of singlet MWCNT in diaphragm was comparable to that of singlet MWCNT in the lungs 5.6 ± 0.6 versus 5.1 ± 0.6 μm, respectively. There were 26 ± 13 and 134 ± 25 per cm² in tissue sections of diaphragm at 1 day and 48 weeks post exposure, respectively. On average, there were 18 ± 5 and 50 ± 20 per cm² singlet MWCNT observed in systemic organ tissue sections at 1 day and 48 weeks, respectively. The burden of singlet MWCNT in parietal pleura, respiratory musculature and systemic organs at 48 weeks post exposure was significantly higher than at 1 day post exposure. Results demonstrate that inhaled MWCNT, which deposit in the lungs, are transported to the parietal pleura, the respiratory musculature and the systemic organs in a singlet form and accumulate with time following exposure.

PS 453 Genotoxicity of Long, Tangled Carbon Nanotubes in Mice.

J. Catalán^{1,2}, H. Järventaus¹, S. Suhonen¹, K. Siivola¹, C. Moreno², E. Rossi¹, J. Koivisto¹, E. Vanhala¹, H. Wolff¹, H. Alenius¹, K. Savolainen¹ and H. Norppa¹. ¹Nanosafety Research Centre, Finnish Institute of Occupational Health, Helsinki, Finland; ²University of Zaragoza, Zaragoza, Spain.

Long, needle-like multiwalled carbon nanotubes (MWCNTs) have been described to induce inflammation, genotoxic effects and mesothelioma in the respiratory system of mice, but the mechanisms behind these adverse effects are not well understood. The stiffness of the CNTs has been suggested to play a crucial role in their clearance from the lungs, affecting their toxicity. We have earlier observed that long, needle-like MWCNTs increase DNA damage in murine lungs. To find out whether the shape of the CNTs could affect their genotoxic properties, we examined here whether also long, but tangled MWCNTs (outer diameter 8-15 nm; Cheaptubes Inc), administered either by pharyngeal aspiration or inhalation, could be genotoxic in C57BL/6J mice locally in the lungs or systemically in peripheral leukocytes and bone marrow erythrocytes. Cell samples were collected 24-h after a single pharyngeal aspiration (0.02-4 mg/ml) or a 4-day inhalation exposure (4 h/day; 17.5 mg/m³) to the MWCNTs. DNA damage was assessed by the comet assay in bronchoalveolar lavage (BAL) cells and lung cells. DNA double strand breaks were assessed by the γ-H2AX assay in peripheral leukocytes and (after pharyngeal aspiration) in lung cells. Micronuclei, a biomarker of chromosome damage, were analyzed in bone marrow polychromatic erythrocytes sampled 24 h after the end of the inhalation exposure. No significant dose-dependent increase in DNA damage (comet assay) was seen in the BAL or lung cells of mice treated by pharyngeal aspiration or by inhalation exposure. The long, tangled MWCNTs neither induced systemic genotoxic effects in peripheral leukocytes or bone marrow. Our findings suggest that the stiffness of long MWCNTs is a central characteristic with respect to their genotoxicity in vivo, with thinner and flexible tangled MWCNTs, which tend to form agglomerates, showing no genotoxic effects. (Funded by the Finnish Work Environment Fund)

PS 454 High-Fat Diet Leads to Increased Lung Inflammation and Airway Resistance following Multiwalled Carbon Nanotubes Exposure.

T. A. Brown. Center for Environmental Health Sciences, University of Montana, Missoula, MT.

Obesity has become a worldwide epidemic responsible in large part for the rising costs of health care. Obesity leads to systemic low-grade inflammation increasing risk for the development of diseases such as diabetes, but the link for respiratory disease is less clear. We investigated the effect of a high fat diet on lung inflammation and lung physiology when exposed to multi-walled carbon nanotubes (MWCNT). Nanomaterials, including MWCNT, are used in an increasing number of consumer products. Given their small dimensions with large surface area and often very unique properties with high deposition efficiency they can induce significant immune responses in the lung. In this study, C57Bl/6 mice were kept on a high fat diet for 6 weeks and then exposed to MWCNT, via oropharyngeal instillation. Measurements were taken 24 hr later to determine changes in inflammation and respiratory physiology, specifically lung resistance. Mice given particle on the high fat diet had significantly increased levels of IL-1β, a pro-inflammatory cytokine produced by the inflammatory complex the inflammasome, as well as increased lung resistance compared to mice on the control diet given particle. In order

to further investigate inflammatory changes due to the high fat diet additional studies examined the influx of inflammatory cells in response to MWCNT exposure. Mice on the high fat diet exposed to MWCNT had a greater influx of neutrophils and eosinophils compared to control diet mice exposed to particle. These results indicate that a high fat diet leads to an increase inflammatory response with measurable physiological alterations in the lungs when exposed to MWCNT. This work was supported by NIH grants RC2 ES018742 and P20 RR017670.

PS 455 Multiwalled Carbon Nanotubes Cause Mild Inflammation in the Aorta without Pulmonary Toxicity in a Rapidly Aging Mouse Model.

K. Luyts, S. Smulders and P. Hoet. Environment and Health, Kuleuven, Leuven, Belgium.

Exposure to ambient particulates has been shown to cause co-morbidity in elderly. Brain and muscle ARNT-like protein-1 (Bmal1) clock gene-deficient mice, with an accelerated aging and prothrombotic phenotype, were used to study the pulmonary and cardiovascular toxicity of multiwalled carbon nanotubes (CNTs). At the age of 8 weeks, wildtype and knockout Bmal1 mice were oropharyngeally aspirated once weekly during 5 consecutive weeks with 6.4 μg (32 μg in total), 25.6 μg (128 μg in total) of CNTs or the vehicle as control.

Cell counts in the bronchoalveolar lavage fluid indicated no inflammatory response 24 hours or 2 months after the last aspiration despite the presence of particle-laden macrophages. Cytokine measurements in lung homogenates showed trends for IL-1β, IL-6 and KC increases only in the wildtype mice aspirated with 128 μg CNTs but this response disappeared after 2 months.

In wildtype mice, aspiration of 128 μg CNTs caused a non-significant decrease in platelet and red blood cell counts, no significant differences for the aPTT and PT clotting tests were found and clotting factor FVIII was (non-significantly) decreased 24 hours after the last aspiration and increased 2 months later. In the BMAL1 knockout mice, FVIII was increased after 24 hours but decreased after 2 months.

A macrophage staining (MAC-3) on sections of the aorta showed endothelial activation and vascular inflammation in 60% of the 128 μg dosed knockout animals. There were no changes observed in the aortas of the wildtype mice.

In this study we showed that multiple dosing (5 weekly doses) of CNTs induced a mild vascular inflammation in the high dosed Bmal1 knockout mice in the absence of pulmonary toxicity.

ENPRA Project NMP4-SL-2009-228789

IWT 101061

PS 456 Investigation of the Pulmonary Bioactivity of Double-Walled Carbon Nanotubes.

T. M. Sager¹, M. Wolfarth¹, D. W. Porter¹ and T. Steinbach². ¹NIOSH, Morgantown, WV; ²Experimental Pathology Laboratories, Sterling, VA.

Nanotechnology is one of the world's most promising new technologies. In turn, carbon nanotube production is estimated to reach into the millions of tons within the decade. Our laboratory has previously established that exposure to multi-walled carbon nanotubes (MWCNT) causes lung inflammation and fibrosis in mice after pharyngeal exposure. However, the bioactivity of double-walled carbon nanotubes (DWCNT) has not been determined. In this study we explored the hypothesis that DWCNT would promote pulmonary toxicity by analyzing the pulmonary bioactivity of the DWCNT. To test this hypothesis, male mice (C57BL/6J) were given a single dose of one of the following by pharyngeal aspiration: 1) 0.9% saline with 0.3% (w/v) carboxymethyl cellulose (CMC; vehicle control), or 2) DWCNT (0-40 μg/mouse) suspended in vehicle [0.9% saline with 0.3% (w/v) CMC]. Whole lung lavage (WLL) was conducted at 1 and 7 days post-exposure. Lungs of non-lavaged animals were also collected and processed for histopathologic analysis at 7 and 56 days post-exposure. The results show the DWCNT exposure caused a dose-dependent increase in WLL polymorphonuclear leukocytes, indicating that DWCNT exposure initiates pulmonary inflammation. DWCNT exposure also caused a dose-dependent increase in LDH activity as well as albumin levels in WLL fluid, indicating that DWCNT exposure promotes cytotoxicity as well as decreases in the integrity of the blood-gas barrier in the lung. Also, at 56 days post-exposure, the presence of fibrosis was noted in the highest dose exposure group (40 μg/mouse). In conclusion, this study provides insight into the previously uninvestigated pulmonary bioactivity of DWCNT exposure. The results confirm that DWCNT exposure does promote inflammation and fibrosis in the lung. The results also indicate that DWCNT have a similar pulmonary bioactivity as the previously studied MWCNT.

The Toxicologist

Supplement to *Toxicological Sciences*

52nd Annual Meeting and ToxExpo™

March 10–14, 2013 • San Antonio, Texas



OXFORD
UNIVERSITY PRESS

ISSN 1096-6080
Volume 132, Issue 1
March 2013

www.toxsci.oxfordjournals.org

An Official Journal of
the Society of Toxicology

SOT | Society of
Toxicology

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

www.toxicology.org