PS 1248 Effects of Pristine and Nitrogen-Doped Multiwalled Carbon Nanotubes (ND-MWCNT) on Reactive Oxygen Species (ROS) and Cell Cycle Progression

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ND-MWCNT are modified MWCNT with enhanced electrical properties that are used in a variety of applications including fuel cells and sensors; however, the mode of action of toxicity of ND-MWCNT has yet to be elucidated. Recent *in vivo* data showed that ND-MWCNT induced inflammation and fibrosis in mouse lungs to a lesser extent compared to pristine MWCNT. In this study, we compared the interaction of ND-MWCNT or Mitsui 7 MWCNT (MWCNT-7) with human small airway epithelial cells (SAEC) and evaluated their subsequent bioactivities. ND-MWCNT were characterized by transmission electron microscopy, X-ray photon spectroscopy, and Raman spectroscopy, which suggested the presence of defects in the nanotube lattice. The nanotubes were determined to be 93.3% carbon, 3.8% oxygen, and 2.9% nitrogen. A dose-response MTS assay showed that low doses up to 12 μg/mL of ND-MWCNT and MWCNT-7 increased cellular proliferation, while the highest dose of 120 μg/mL significantly decreased proliferation. ND-MWCNT and MWCNT-7 appeared to be engulfed by SAEC at 6h and were fully internalized by 24h. ROS was elevated at 6 and 24h in ND-MWCNT exposed cells, but only at 6h in MWCNT-7 exposed cells. Significant alterations to the cell cycle were observed in SAEC exposed to either 1.2 μg/mL of ND-MWCNT or MWCNT-7 in a time-dependent manner, as shown by a decreased percentage of cells in S phase and an increased percentage of cells in S and G2 phase, respectively, thus, suggesting potential damage or alterations to cell cycle machinery. Our results indicate that exposure to MWCNT-7 or ND-MWCNT induces effects in SAEC possibly through different mechanisms that are potentially related to physicochemical characteristics that may alter their toxicity.

PS 1249 Cross-Species Approach Reveals ER-Stress As a Conserved Mechanism of MWCNT-Mediated Biological Interactions

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The raised considerable concern about the possible environmental health and safety impact of multi-walled carbon nanotubes (MWCNTs) originated from its potential widespread applications. We performed a comprehensive study about biological interaction of MWCNTs, specifically in regard to its differential surface functionalization (-OH, -COOH, -NH2 and pristine) on Beas2B cells (at various concentrations, 5 to 200 mg/L, for 24h), *Caenorhabditis elegans* (50 mg/L for 24h) and mouse (treated with 1mg/kg with 50 μl of suspension in PBS for 24h). A conserved ER stress mediated biological interactions of MWCNTs were evident in all three model systems but with differential mode of action. The IRE1A –XBP1 pathway mediated ER stress was found in Beas2B cells as well as in mouse and the highest effects were observed in COOH functionalized MWCNTs. Conversely, pek-1 (PERK kinase homologue) pathway was the main mechanism of ER stress in case of *Caenorhabditis elegans* and the pristine MWCNT showed the highest effects in the worms. In summary, our results present a paradigm for analyzing surface functionality-activity relationship of MWCNT with different model systems which could eventually be utilized for more efficient and innocuous applications, specifically in biomedical field.

PS 1250 MRNAs and miRNAs in Whole Blood Associated with MWCNT-Induced Lung Hyperplasia, Fibrosis, and Bronchiolo-Alveolar Adenoma and Adenocarcinoma following Inhalation Exposure in Mice

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Inhalation exposure to MWCNT in mice results in inflammation, fibrosis, and the promotion of lung adenocarcinoma; however, the molecular basis behind these pathologies is unknown. This study determined global mRNA and miRNA profiles in whole blood from mice exposed by inhalation to MWCNT that correlated with the presence of lung hyperplasia, fibrosis, and bronchiolo-alveolar adenoma and adenocarcinoma. Six week old, male, B6C3F1 mice received a single intraperitoneal (i.p.) injection of either the initiator methylcholanthrene (MCA, 10 μg/g BW) or vehicle (corn oil). One week after injections, mice were exposed by inhalation to MWCNT (5 mg/m3, 5 hours/day, 5 days/week) or filtered air (control) for a total of 15 days. At 17 months post-exposure, mice were euthanized and examined for lung pathology, and whole blood was collected and analyzed by microarray for global mRNA and miRNA expression. Numerous mRNAs and miRNAs in the blood were significantly up- or down-regulated in the presence of lung pathology after MCA and/or MWCNT inhalation, including fcrl5 and miR-122-5p in the presence of hyperplasia, mthfd2 and miR-206-3p in the presence of fibrosis, fam178a and miR-130a-3p in the presence of bronchiolo-alveolar adenoma, and il7r and miR-210-3p in the presence of bronchiolo-alveolar adenocarcinoma, among others. These miRNAs and mRNAs and their respective regulatory networks may be correlated with MWCNT-induced lung pathology and may potentially serve as biomarkers for the presence of lung pathology following MWCNT exposure.

PS 1251 Systematic Evaluation of Carbon Nanotube Toxicity Using the Embryonic Zebrafish to Inform Health and Safety

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The unusual physicochemical properties of carbon nanotubes (CNTs) make them attractive for applications in sporting goods, electronics, water purification and biomedicine. With global CNT production predicted to surpass 12,300 metric tons in 2015, there is increased likelihood of unintended environmental impacts and a need to systematically characterize how CNT physicochemical traits contribute to adverse biological response. We exposed eight-hour post fertilization (hpf), dechorionated embryonic zebrafish to five-fold dilutions of systematically modified multi-walled carbon nanotubes (MWNTs) for five days and assessed zebrafish for morphological malformations and mortality at 24 and 120 hpf. MWNTs varied by charge, percent surface oxygen, aggregate radius, aggregate morphology and electrochemical activity. Mortality at 24 hpf was the primary adverse response observed in our assay and only occurred with any significance at 50 mg/L. We employed multivariate statistics to develop a predictive MWNT toxicity model based on that data. Mortality positively correlated with dispersion of MWNTs and modeling identified surface charge as the primary predictor of zebrafish mortality. A validation set of MWNTs from a different vendor and systematically treated in the same manner confirmed the model. The predicted mortality of the test MWNTs generally agree with the actual results. Incorporation of transcriptional and proteomic data associated with MWNT exposure will improve model predictions and inform safer CNT design. Research support: NIH grant T32 ES07060 and P30 ES000210 and U.S. EPA Assistance Agreement RD83558001-0. This abstract has not been reviewed by the EPA and any views expressed are solely those of the authors and do not necessarily reflect those of the Agency.

PS 1252 Uptake, Translocation, and Stress Effects of Carbon Nanotubes in Drought-Induced Corn

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Carbon nanotubes are one of the most used manufactured nanomaterials. However, these materials are not regulated and there are concerns regarding their behavior in the environment and human health. This study was conducted to evaluate uptake of various types of carbon nanotubes in corn under ideal watering and drought conditions. Corn was exposed to either non-functionalized carbon nanotubes (CNTs) or functionalized carbon nanotubes (COOH-CNTs). Plants were grown for 21 days in soil with 10 mg/kg of CNTs or COOH-CNTs in1 L or 3 L of soil in a greenhouse with natural day:night conditions. Corn was also grown under conditions simulating a seven-day drought and photosynthesis measurements were taken using a LI-6400XT Portable Photosynthesis System. Following harvest after 28 days, roots, stems, and leaves were dried, grounded, and analyzed using a microwave-induced heating technique to quantify CNT and COOH-CNT concentrations in the corn. Photosynthetic rate declined throughout the duration of the drought treatments. CNT uptake was only detected in roots of drought-treated plants exposed to CNTs and COOH-CNTs. Additional plant analyses are currently ongoing.

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