PS 1240 *In Vivo* Evidence of Free Radical Formation in the Mouse Lung and Distant Organs after Exposure to Single-Walled Carbon Nanotubes

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Previously, we have reported that pharyngeal exposure of C57BL/6 mice to single walled carbon nanotubes (SWCNTs) caused formation of granulomatous bronchial interstitial pneumonia, fibrosis, oxidative stress, acute inflammatory/cytokine responses and a decrease in pulmonary function. In the current study, we used electron spin resonance (ESR) to directly assess whether pulmonary exposure to respirable SWCNTs caused formation of free radicals in the lungs and in two distant organs, the heart and liver. Here we report that exposure to partially purified SWCNTs (HiPco, CNI, Inc, TX) resulted in the augmentation of oxidative stress as evidenced by ESR detection of a-(4-pyridyl-1-oxide)-N-tert-butylnitrone (POBN) spin-trapped carbon-centered lipid-derived radicals recorded shortly after the treatment. This was accompanied by a significant depletion of antioxidants and elevated biomarkers of inflammation presented by recruitment of inflammatory cells and an increase in pro-inflammatory cytokines in the lungs, as well as development of multifocal granulomatous pneumonia, interstitial fibrosis and suppressed pulmonary function. Moreover, pulmonary exposure to SWCNTs also caused the formation of carbon-centered lipid-derived radicals in the heart and liver at later time points (day 7 post exposure). Additionally, SWCNTs induced a significant accumulation of oxidatively modified proteins, an increase in lipid peroxidation products, depletion of antioxidants and an inflammatory response in both the heart and the liver. Overall, we provided direct evidence that lipid-derived free radicals are a critical contributor to tissue damge induced by SWCNTs not only in the lungs, but in distant organs.

PS 1241 Comparative Inhalation Toxicities of Graphene and Other Carbonaceous Nanomaterials

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Graphene (G), graphite nanoplatelets (GP), carbon nanotubes (mwCNT) and low surface area carbon black (CB) are carbon-based nano-materials with broad technological applications. mwCNT and CB possess different inhalation toxicities, whereas less is known about G and GP. In order to compare the inhalation toxicity of these carbon-based nanomaterials, male Wistar rats were exposed head-nose to aerosols for 6h/day on 5 consecutive days. Target concentrations were 0.1, 0.5, or 2.5 mg/m3 for mwCNT and 0.5, 2.5, or 10 mg/m3 for G, GP and CB. Toxicity was determined at the end of exposure and three week later using broncho-alveolar lavage fluid and microscopic examinations of the entire respiratory tract. No adverse effects were observed after inhalation exposure to 10 mg/m3 GP or CB. Increases of lavage markers indicative for inflammatory processes started at exposure concentration of 0.5 mg/m3 for mwCNT and 10 mg/m3 for G. Consistent with these changes, granulomateous inflammations were observed at 2.5 mg/m3 mwCNT and G. In order to evaluate volumetric loading of the lung as the key parameter driving the toxicity, deposited particle volume was calculated, taking into account different methods to determine the agglomerate density. However, the calculated volumetric load did not correlate to the toxicity, nor did the particle surface burden of the lung. The inhalation toxicity of carbon-based materials is likely to be a complex interaction of several parameters. Until the properties which govern the toxicity are identified, testing by short-term inhalation is the best option to identify hazardous properties in order to avoid unsafe applications or select safer alternatives for a given application. Ma-Hock, Lan, et al. "Comparative inhalation toxicity of multi-wall carbon nanotubes, graphene, graphite nanoplatelets and low surface carbon black." *Particle and fibre toxicology* 10.1 (2013): 23.

PS 1242 Inhalation Toxicity of Carbon Black Depends on Surface Coating with Polycyclic Aromatic Hydrocarbons

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The aim of this study was to assess differences in the toxic potential of surface modified carbon black particles. To this end a 14-day inhalation study was conducted in rats using nose-only exposure to compare the effects of pristine Printex®90 with surface modified carbon black, i.e. acetylene soot particles and Printex®90 coated with benzo[a]pyrene. This set of particles was also tested in different *in vitro* and *ex vivo* systems. On day one after the end of the 14-day inhalation period, acetylene soot alone caused an increase in relative lung wet weights. Furthermore, acetylene soot caused the most frequent histological alterations like interstitial inflammatory cell infiltration and bronchiolo-alveolar hyperplasia. Cytotoxicity tests with human pulmonary cell lines and precision cut lung slices (PCLS) were limited due to solubility of compounds. However, measurement of the transepithelial electrical resistance (TEER) in Calu-3 cells as well as the analysis of reactive oxygen species (ROS) in A549 and 16HBE14o- cells were able to differentiate between the carbon black modifications. Murine *ex vivo* airway preparations also proved to be a valuable model as acetylene soot was found to be the most toxic carbon black modification for epithelial cells, and the mechanism of action was linked to CYP1A1 induction. In summary, the results of this study suggest that the acute inhalation toxicity of carbon black is low, but increased if the surface is coated with polycyclic aromatic hydrocarbons. *In vitro* models with proven CYP1A1 inducibility seem to be useful tools to predict the *in vivo* effects of carbon black, coated with polycyclic aromatic hydrocarbons, on pulmonary epithelia.

PS 1243 Single-Wall or Double-Wall Carbon Nanotubes Induce Atherosclerosis Progression in Animal and Culture Models of Atherosclerosis

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Background: Recently, the use of carbon nanotubes has been increasing rapidly in a variety of fields. However, cardiovascular effect of exposure to carbon nanotubes remains elusive. The present study investigated the effects of pulmonary exposure to single-wall carbon nanotubes (SWCNT) or double-wall carbon nanotubes (DWCNT) on atherosclerosis progression in normal human aortic endothelial cells (HAECs) and apolipoprotein E (ApoE) null mice, a widely used model for human atherosclerosis. Methods and results: ApoE null mice were exposed by pharyngeal aspiration to SWCNT or DWCNT (10 or 40 μg/mouse) once every other week for 10 weeks. Oil red O staining showed increase in the plaque areas of the aorta in ApoE null mice exposed to SWCNT or DWCNT compared with the vehicle-treated ApoE null mice. The expression of the adhesion molecule (ICAM-1) was increased on the aorta in ApE null mice exposed to SWCNT or DWCNT. Since it is known that the endothelial progenitor cells (EPCs) are mobilized from bone marrow into the circulation and subsequently migrate to the site of endothelial damage and repair, we isolated the EPCs from bone marrow of exposed mice and analyzed their *ex vivo* function. Seven days after the end of exposure, colony-forming units and migration assays were performed. Exposure to SWCNT or DWCNT at high dose reduced colony-forming units. Moreover, exposure to SWCNT at high dose decreased the number of migration cells. HAECs were cultured and exposed to carbon nanotubes. High-does SWCNT or DWCNT reduced cell viability, increased the expression of ICAM-1, and enhanced adhesion of the THP-1 monocyte to HAECs. Conclusion: The study suggested that SWCNT and DWCNT induced atherosclerosis progression through the reduction of the function of EPCs and enhancement of adhesion of monocytes to endothelial cells.

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