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.

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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 ug/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 ug/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 ($p \leq 0.05$; 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.

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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.

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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.

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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 (transmition 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, chromatine 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, serpina4]), 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.

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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/m^3 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,

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