

since GD35 until parturition. Control animals received no experimental treatment. Placental tissue was collected at parturition for histopathological and LHT evaluation. Placental tissue from experimental rats showed labyrinth zone thickening and reduction of the junctional zone thickness, LHT revealed accumulation of sugars in some cells located at several regions of the placenta. Placental tissue from experimental goats showed cytoplasm vacuolization in columnar epithelium of chorioallantoic membrane, LHT revealed higher markup for the SWGA and WGA lectins suggesting that the vacuolated cells contain b-(1-4)-N-acetylglucosamine, and N-acetylneuramic acid in vacuoles, one of the main carbohydrates accumulated in this toxicosis. The results clearly revealed that the placental tissue is also target of toxic action of the toxic active ingredients present in the IC. Probably, changes in fetal development observed in these two animal species exposed to IC are also a consequence of the injury in the placental tissue that inevitably causes losses to the developing fetus. *Financial supported by: FAPESP (2006/58729-2).*

PS 2703 Quantitative Comparison of Pristine and Carboxylated Carbon Nanotubes Accumulation and Cell Surface Binding on Macrophages

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The global increase in the production of carbon nanotubes (CNTs) and incorporation into consumer products causes concern for CNTs toxicity. Macrophages are a key intermediary in CNT pathology and the molecular mechanisms of how CNTs influence inflammatory responses have been widely studied; however, there remain significant knowledge gaps and conflicting literature reports on identifying CNTs receptors on cells. One reason for this gap is the challenge of accurately measuring the accumulation of various CNTs in cells. Further, the lack of tool for measuring the small amounts of CNTs associated with cells in direct surface binding assays hamper defined studies on the interaction of CNTs with receptors. First, we applied the CNT detection methods developed previously for epithelial cells and zebrafish embryos to study the accumulation of pristine and carboxylated CNTs in macrophages. Preliminary results shown that mouse RAW264.7 and differentiated human THP-1 macrophages accumulated 80-100 times more carboxylated multi-walled carbon nanotubes (C-MWNTs) and single-walled carbon nanotubes (C-SWNTs) than corresponding pristine MWNTs (P-MWNTs) and SWNTs (P-SWNTs) suspended in cell culture medium with the triblock copolymer Pluronic® F-108 during a 24 hour exposure at 37 °C. The selective accumulation of C-MWNTs and C-SWNTs compared to pristine material suggests that there are receptors that preferentially bind carboxylated CNTs compared to pristine CNTs. To test for binding, macrophages were incubated with C-MWNTs or P-MWNTs in medium without fetal bovine serum (FBS) at 4 °C, conditions that avoid any interference by cell processes active at physiological temperature, such as endocytosis, or by FBS proteins. There was strong binding of C-MWNTs and C-SWNTs to cells while the binding of P-MWNTs and P-SWNTs was not significant, consistent with the selective accumulation of C-MWNTs and C-SWNTs at 37 °C. These data provide strong evidence for abundant receptor sites on cells that bind carboxylated CNTs but not pristine material. Further, the binding of carboxylated CNTs to cells was reduced when the medium contained FBS, suggesting that there are FBS components that compete with carboxylated CNTs for the surface receptors to reduce binding, which may help identify the receptors in future studies.

PS 2704 The Convergence of Fibrogenic and Estrogen Receptor Signaling Pathways: A Target for Multi-Walled Carbon Nanotubes

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Pulmonary fibrosis (PF) is an irreversible condition that if progressive can lead to potentially fatal lung disease characterized by organ malfunction and respiratory failure. PF can have unknown etiology as in idiopathic pulmonary fibrosis (IPF) or be associated with exposure to environmental agents such as asbestos fibers and potentially multi-walled carbon nanotubes (MWNT). The role of sex hormones and their receptors in PF is unclear despite the existence of sex-specific trends in the incidence and prevalence of IPF. The goal of this research is to examine a role for estrogen (E2) in fibrogenic signaling induced by MWNTs in bronchial epithelial cells (BEAS-2B). BEAS-2Bs were exposed to Mitsui MWNTs and estrogen receptor alpha (ESR1) gene expression was measured by qPCR. Results revealed a statistically significant ($p < 0.05$) 16, 48, and 56% reduction in ESR1 mRNA expression after 48 hr exposure to 0.2, 2, and 20 µg/mL MWNTs, respectively. Inhibition of transforming growth factor beta1 (TGF-β1), a pro-fibrogenic cytokine shown to be up-regulated by MWNTs, using a receptor-specific inhibitor blocked the

MWNT-induced reduction suggesting a role for TGF-β1 in mediating the inhibitory effects. Next we performed an RNAseq analysis of BEAS-2Bs exposed to E2 in the presence and absence of TGF-β1 to identify downstream targets of E2 that are susceptible to TGF-β1 and potentially MWNTs. Results revealed distinct expression profiles where exposure to TGF-β1 resulted in modulation of genes involved in extracellular matrix organization and cell adhesion by E2. Of the genes involved in cell adhesion, Muc15 emerged as a potential target of E2 as Muc15 exhibited a statistically significant ($p < 0.05$) Log₂(Fold Change) of 2.09 in the E2 group, -4.61 in the TGF-β1 group, and no statistically significant difference in the group co-exposed to TGF-β1 and E2 suggesting that TGF-β1 inhibited the E2-induced expression through inhibition of ESR1 gene expression. Current work is investigating Muc15 as a target for MWNTs. Results of this work highlight ESR1 and E2 target genes as potential targets of MWNTs in the lung.

PS 2705 Classification of Chemical Reactivity of Various Carbon Nanotubes Using Redox Potential to Estimate ROS Toxicity from Physicochemical Properties

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The redox potential is an important physicochemical property to estimate toxicity of nano materials because most bioactivities are redox reactions. Recently, as far as nano particle reactions in the endoplasmic compartment are concerned, interactions among nano particles, proteins, and biomarkers must be explored with respect to redox potentials. Redox potentials of carbon nanotubes have been discussed previously; however, they have not been evaluated in detail as yet. The present study systematically examined the kinetics of a hydroxyl radical scavenging reaction for various carbon nanotubes (CNTs), including double-walled and multi-walled carbon nanotubes (DWCNTs and MWCNTs), and carbon nano peapods (AuCl₃@DWCNT). A previously proposed theoretical model was used to analyze the experimental results. The reaction kinetics for DWCNTs and thin MWCNTs agreed well with the theoretical model and were consistent with each other. Furthermore, thin and thick MWCNTs behaved differently, which also was consistent with the theory. Additionally, surface morphology of CNTs substantially influenced the reaction kinetics, while addition of doped particles into the hollow center of CNTs (AuCl₃@DWCNT) shifted the redox potential in a different direction. These findings make it possible to predict the chemical and biological reactivity of CNTs based on their structural and chemical nature and their redox potential. (*ST was partially supported by the research program "Center of Innovation" by Japan Science and Technology Agency*)

PS 2706 High-Dimension Biological Analysis of Multi-Walled Carbon Nanotube Toxicity

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The toxic effects of multi-walled carbon nanotubes (MWCNTs) are often associated with intracellular oxidative processes related to generation of reactive oxygen species (ROS). The latter is enhanced by metallic impurities, that can be mobilized from the carbon nanotubes to the surrounding biological microenvironment. Aiming at identifying the effects of exposure to purified and unpurified MWCNTs on different biological targets immunological, biochemical, gene expression and biological pathway analyses were carried out on samples from health human volunteers and *in vitro* models. The MWCNTs with higher amount of metallic impurities caused a 1.8-fold increase in lipid hydroperoxide levels, while the number of mature T lymphocytes with reduced potential underwent a more than 3-fold increase, as compared to the purified MWCNTs. Whole genome transcriptomics in lung epithelium cells (A549) and keratinocytes (HaCaT) showed that gene expression is significantly modulated after exposure to purified and unpurified MWCNTs. 48-hour exposure resulted in approximately 500 genes expressed differentially in the case of MWCNTs with different levels of impurities. Similar results were found when we analyzed the data isolating the 25 genes involved in the molecular pathway associated to oxidative stress. The persistence of the induction of genes driving chemokine and cytokine-signaling-mediated inflammation showed the importance of the inflammatory effects of MWCNTs, and in particular the unpurified ones. Pathway analysis showed significant modulation of genes related to the NFκB pathway, after exposure to unpurified MWCNTs, as a result of oxidative stress induction. This may cause a perturbation of the IL-6



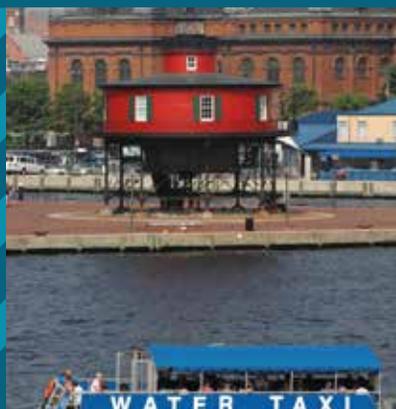
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Supplement to *Toxicological Sciences*



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Baltimore, Maryland | March 12–16, 2017



OXFORD
UNIVERSITY PRESS

ISSN 1096-6080
Volume 156, Issue 1
March 2017

www.toxsci.oxfordjournals.org



The Official Journal of
the Society of Toxicology

SOT | Society of
Toxicology

www.toxicology.org

