their bioactivities. Previous studies have shown that pulmonary exposure to dispersed SWCNT, caused a greater interstitial lung fibrosis in mice than the non-dispersed form. In this study, we further investigated the effect of nanoparticle dispersion on cellular activities in vitro using a natural lung surfactant (Survanta®) as a dispersing agent. Human bronchial epithelial BEAS-2B cells and human lung fibroblast CRL-1490 cells were exposed to physiologically relevant concentrations of SWCNT (0.02-0.6 μg/cm2) with or without Survanta®(150μg/ml) and their effects on cell viability, proliferation, and collagen production were determined by LDH assay, cell counting, and Western blot analysis, respectively. The results showed that: 1) Survanta® was effective in dispersing micron-sized SWCNT agglomerates to nano-sized structures; 2) Survanta® when used alone had no significant effect on the measured cellular activities; 3) Survanta®-dispersed SWCNT exhibited a biphasic effect on cells inducing proliferation at low doses and causing toxicity at high doses, while non-dispersed SWCNT had no significant effects; 4) In lung fibroblasts, dispersed SWCNT upregulated collagen expression, whereas non-dispersed SWCNT had a lesser effect. These results are supported by in vivo data and suggest that dispersed SWCNT is more fibrogenic than non-dispersed SWCNT. Due to the rapidity and simplicity of the in vitro assay models described in this study, this model could potentially be used as a rapid screening tool for fibrogenicity and toxicity testing of nanoparticles.

PS 798 GRAPHENE INDUCED CYTOTOXICITY AND OXIDATIVE STRESS: AN *IN VITRO* STUDY.

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The unique structure and surface properties of graphene make this carbon-based nanomaterial a good candidate for mammalian and microbial detection. Little is known about the potential toxic effects of this nanomaterial. Therefore, we determined if graphene produces toxicity in PC12 cells. Graphene was characterized using Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM) and Confocal Raman Microscopy. High-resolution TEM images revealed that the graphene samples are composed of graphene sheets overlaid side-by-side (1-5 layers), with dimensions of 100 nm in diameter. Graphene produces reactive oxygen species (ROS) in a concentration- (0-100 ug/ml) and time-dependent manner. LDH assays for cell death and MTT assays for cell viability were performed and the results indicated that graphene produces concentration-dependent cytotoxicity. Furthermore, caspase 3, also a marker of oxidative stress, was also activated in a concentration-dependent manner after exposure to graphene, suggesting graphene produces apoptosis. Interestingly, dopamine (DA) levels in the cells increased in a concentration-dependent manner at 24 hours as measured by HPLC/EC. Together, our data suggest that graphene, like many other carbon-based nanomaterials such as carbon nanotubes and fullerene, exhibit oxidative potential in a biological system. Support by NCTR E7282 and ORISE.

PS 799 INDUCTION OF MITOTIC SPINDLE ABERRATIONS BY OCCUPATIONALLY RELEVANT DOSES OF SINGLE-WALLED CARBON NANOTUBES.

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Engineered carbon nanotubes are newly emerging manufactured particles with potential applications in electronics, computers, aerospace and medicine. The low density and small size of these biologically persistent particles makes respiratory exposures to workers likely during the production or use of commercial products. We have previously shown mitotic spindle aberrations in cultured primary and immortalized human airway epithelial cells exposed to 96, 48 and 24 micrograms/centimeter squared single-walled carbon nanotubes (SWCNT). To investigate mitotic spindle aberrations at concentrations anticipated in exposed workers, primary and immortalized human airway epithelial cells were exposed to SWCNT for 24-72 hours. We have now demonstrated fragmented centrosomes, multiple mitotic spindle poles and aneuploid chromosome number at doses equivalent to the permissible exposure limit. The nanotube bundles are similar to the size of microtubules that form the mitotic spindle and may be incorporated into the mitotic spindle apparatus. Confocal microscopy demonstrated nanotubes within the nucleus and in association with mitotic tubulin, the centrosomes and the chromatin in cells exposed to 2.4, 0.24 and 0.024 micrograms/centimeter squared SWCNT. The lower doses do not cause toxicity by Alamar Blue assay, apoptosis by TUNEL or reduction in colony formation after 24 hours. However, after 3 days, the colony formation of the primary cells was reduced. Our results show significant disruption of the mitotic spindle by SWCNT at occupationally relevant doses. Centrosome fragmentation, mitotic spindle disruption and aneuploidy are characteristics of cancer cells and may lead to an increased risk of cancer. *The authors contributed equally to the work.

PS 800 PULMONARY TOXICITY ASSESSMENT OF MULTI-WALL CARBON NANOTUBES *IN VITRO*.

A. Mishra[1, 2], V. Castranova[1, 2], Y. Rojanasakul[2], J. Hall[2] and L. Wang[1, 2]. *[1]HELD/PPRB, National Institute Occupational Safety and Health, Morgantown, WV and [2]School of Pharmacy, West Virginia University, Morgantown, WV.*

Multi Walled Carbon nanotubes (MWCNT) are one of the most popular nanomaterials as reflected by their wide range of applications in electronics, material science and biomedical research. However, their fiber-like structure, low specific weight and nanoscale dimension also raises concerns about possible adverse effects on human health and the environment. In this study, physiologically relevant doses (0.06-0.2 μg/cm2) of MWCNT, which have been shown to cause pulmonary disorders in mice, were examined for their cellular toxicity and inflammatory and fibrogenic cytokine production in human bronchial epithelial BEAS-2B cells and alveolar epithelial A549 cells. MWCNT were dispersed in natural lung surfactants and exposed to the cells in culture. Cellular toxicity was assessed over time by direct cell counting and by WST proliferation assay. Inflammatory/fibrogenic TGF-β and MMP-9 production was determined by Western blot assay. The results showed that: 1) MWCNT can be effectively dispersed using natural lung surfactants, 2) Dispersed MWCNT caused a substantial decrease in cell viability and proliferation as compared to non-dispersed MWCNT or vehicle control treatment, 3) Dispersed MWCNT also induced TGF-β and MMP-9 upregulation in the lung epithelial cells, indicating their fibrogenicity and inflammation potential which is also supported by our in vivo studies. Thus, the in vitro methods may potentially be used as a predictive model for in vivo toxicity assessment and to aid the mechanistic studies of nanomaterial-induced pulmonary toxicities.

PS 801 CHARACTERIZATION OF THE SURFACE ADSORPTION PROPERTIES OF MULTI-WALLED CARBON NANOTUBES IN BIOLOGICAL CONDITIONS *VIA* QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP (QSAR).

Y. Chen, X. Xia, N. A. Monteiro-Riviere and J. E. Riviere. *Center for Chemical Toxicology Research and Pharmacokinetics, North Carolina State University, Raleigh, NC.*

Carbon nanotubes have been studied extensively for drug delivery and potential toxicity in occupational and environmental exposures. Once entering biological systems, numerous biological components will interact with the surface of carbon nanomaterials. There is no method available to quantitatively measure these interactions. Here, we report a QSAR approach to characterize the surface adsorption properties of multiwalled carbon nanotubes (MWCNT), in which a set of small molecules having diverse physicochemical properties was used as probe compounds. The adsorption coefficients (k) of the probe compounds were obtained by measuring the quantities of the probe compounds adsorbed on the surfaces of the nanomaterials and the equilibrium concentrations of the probe compounds in the media. The log (k) values were scaled to a set of solvation molecular descriptors of the probe compounds via multiple linear regressions to provide a set of five nano-descriptors representing the contributions of the five types of molecular interactions: hydrophobicity (V), hydrogen-bond acidity (α) and basicity (β), dipolarity/polarizability (π), and lone-pair electrons (R). A QSAR model was established for MWCNT using the five nano-descriptors; $\log(k) = -1.33 + 0.043R + 1.75\pi - 0.37\alpha - 2.78\beta + 4.18V$, $n=28$, $R^2=0.93$, $F=63$ with significance of 2.7x10-12. The nano-descriptors provided a better correlation ($R^2=0.93$) with adsorption coefficients than hydrophobicity (logKow) alone ($R^2=0.57$) (Supported by U.S. EPA STAR Grant # R833328 and USAFOSR Grant # FA9550-08-1-0182)

PS 802 NOVEL MOLECULAR PATHWAYS INDUCED IN FUNCTIONALIZED FULLERENE EXPOSED HUMAN EPIDERMAL KERATINOCYTES AND HUMAN BRONCHIAL EPITHELIAL CELLS.

J. Gao and R. Iyer. *LANL, Los Alamos, NM.*

Engineered nanomaterials have been extensively used for diagnostics and therapeutics based on their unique physicochemical properties. Since native C60 is hydrophobic, many hydrophilic C60 fullerene derivatives have been synthesized to fa-

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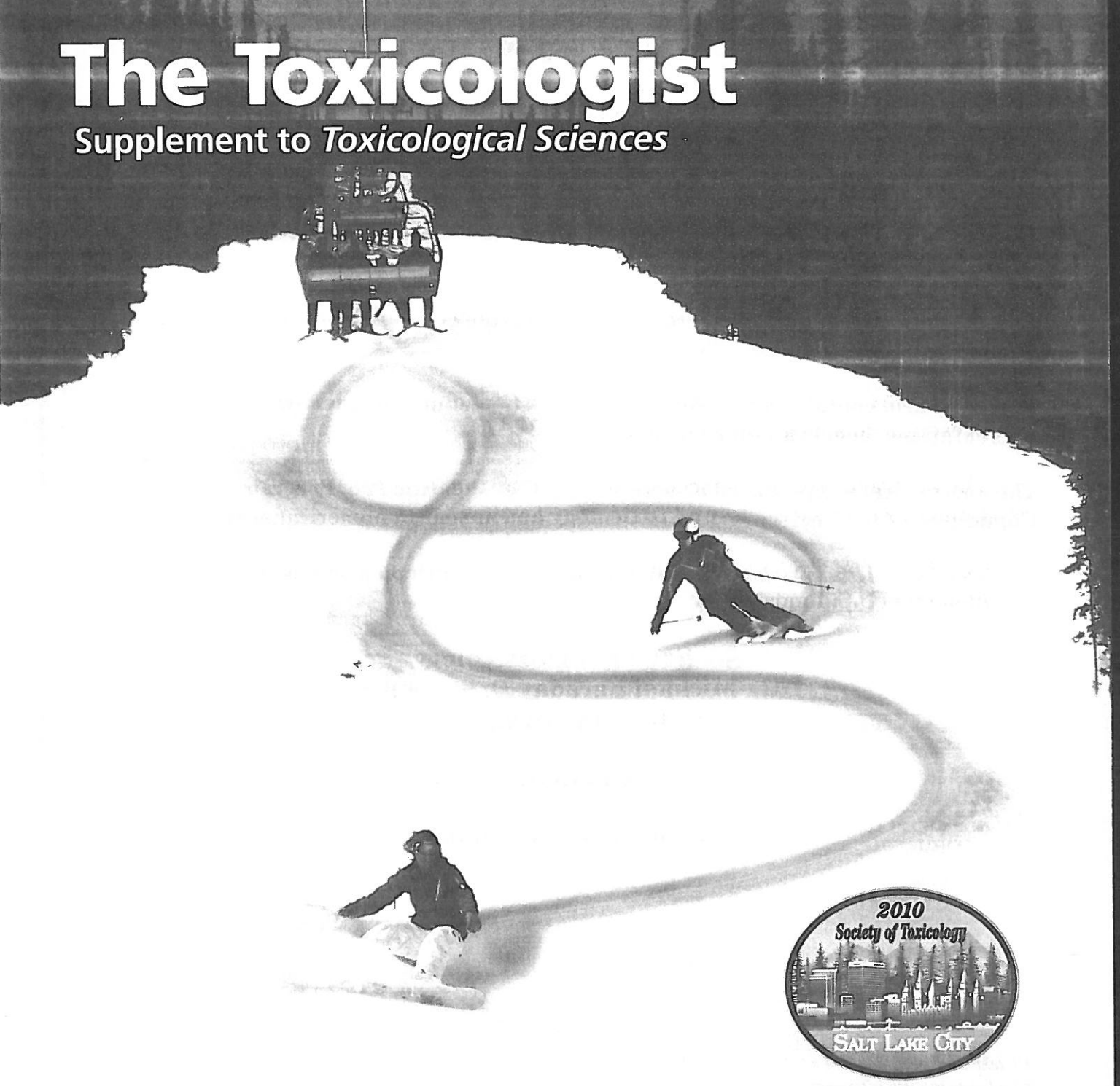

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 49th Annual Meeting of the Society of Toxicology, held at the Salt Palace Convention Center, March 7–11, 2010.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 473.

The issue also contains a Key Word Index (by subject or chemical) of all the presentations, beginning on page 496.

The abstracts are reproduced as accepted by the Scientific Program Committee of the Society of Toxicology and appear in numerical sequence.

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