posure. Antibodies against TNFα, IL-1β and IL-6 were added together with the SWCNT to evaluate its role in the expression of MIP-1α and MIP-1β in Tricultures+EAHY926. The results were expressed as fold changes compared to unexposed cultures. RESULTS. Single cultures: IL-1β was induced in THP-1 cultures (3 fold) while IL-8 was induced in HMC-1 cultures (3 fold). Bicultures: Increases of TNFα (4 fold), IL-1β (2.5 fold) and IL-6 (2.5 fold) were observed in A549+THP-1 Bicultures. Tricultures: No changes in any of the evaluated cytokines were observed when Tricultures were exposed, but increases in MIP-1α (1.5 fold) and MIP-1β (2 fold) were found when EAHY926 cells were introduced in the system. Anti IL-1β enhanced the expressions of MIP-1α (13 fold) and MIP-1β (20 fold). CONCLUSIONS. IL-1β seems to play an important role in regulating the expression of MIP-1α and MIP-1β when pneumocytes+macrophages+mast cells exposed to SCWNT are in communication with endothelial cells.

PS 2211 FUNCTIONALIZATION-DEPENDENT CYTOTOXICITY OF SINGLE- AND MULTI-WALLED CARBON NANOTUBES.

L. Manzo[1, 2], E. Roda[1], D. Sarigiannis[3], G. Cimino-Reale[3], P. Mustarelli[1], A. Profumo[1] and T. Coccini[1, 2]. [1]*University of Pavia, Pavia, Italy,* [2]*Department of Toxicology, Maugeri Foundation Hospital, Pavia, Italy and* [3]*Joint Research Centre, European Commission, Ispra, Italy.*

Recently developed functionalization schemes have extended the application spectrum of carbon nanotubes (CNTs) enhabling the implementation of new functions that cannot otherwise be acquired by pristine nanotubes. Functionalization has been shown to impact on the biological response to CNTs suggesting that toxicological profile may also be modified. In this study, the cytotoxicity of single-walled CNTs (SW), multi-walled CNTs (MW) and functionalized MW (MW-COOH, MW-NH2) was investigated in human astrocytoma D384 and lung carcinoma A549 cell cultures using MTT assay and calcein/propidium iodide (PI) staining. The test nanomaterials were characterized by thermal analysis (TGA), infrared spectroscopy, and atomic force microscopy to assess the degree of purity and functionalization. The cells were exposed to the CNTs (0.1-100μg/ml) for 24, 48, 72h in medium containing 10%FCS. Quartz (SiO2) and carbon black were also similarly tested. In both cell types, MTT data revealed strong cytotoxicity (50% loss of cell viability) of SW after 24h-exposure already at 0.1μg/ml, without further changes at higher concentrations or longer incubation times. At all time-points MTT metabolism was decreased by about 50% by all the other compounds at 10μg/ml and with no exacerbation at the higher dose. Parallel assays using calcein/PI staining did not confirm MTT cytotoxicity data neither in D384 nor in A549 cells. Cell viability was not affected by any CNT type at any concentration or time of exposure, whereas extensive cell killing was observed in the preparation exposed to the positive control SiO2. The results indicate that CNTs can interact with certain types of dye markers that are commonly used in cell culture experiments. This may lead to artefactual data. A study design using multiple tests is recommended in investigations examining CNT toxicity in vitro (Grants from the Italian Ministries of Health, Education and Research).

PS 2212 THE IMPORTANCE OF CELL TYPE FOR *IN VITRO* TESTING OF CARBON NANOFIBERS USING CELL COLONY FORMATION AS ENDPOINT.

T. Syversen, K. Gellein, S. Hoel and L. Evje. *Department of Neuroscience, Norwegian University of Science and Technology, Trondheim, Norway.*

Carbon nano materials are difficult to test by standard in vitro cytotoxicity tests as they may interfere with colorimetric assays. A range of carbon nano tubes as well as carbon particles have been tested using several cell types (RBE4, A549 and NHK3025) for their ability to inhibit cell colony formation and development. Carbon fibers were added to cell cultures prior to, simultaneously or soon after seeding cells at low concentration. Fiber samples were added as suspended by thorough mixing or repeated sonication at low energy. After appropriate exposure the cultures are stained with Giemsa and analyzed. A number of indicators can easily be recorded, e.g. number and size of colonies, number of cells in a colony as well as other morphological characteristics. The test will differentiate between particles like single wall carbon nanotubes (SWCNT), carbon black and even different batches of chemically equivalent carbon particles. Samples were tested in the concentration range of 0.5 to 20 mg per litre. Plating efficiency was reduced by 30-90% in a dose-response manner and differed considerably between the cell types tested. We found that RBE4 cells were the most sensitive cells in this assay and effects could be observed at concentrations as low as 1 mg per litre and the test could differentiate between different production batches of carbon fibers.

PS 2213 A COMPARATIVE STUDY OF EFFECTS OF SINGLE-WALL CARBON NANOTUBES AND CROCIDOLITE ASBESTOS IN HUMAN BEAS-2B CELLS.

M. Pacurari, V. Castranova, S. Friend, V. Robinson and V. Vallyathan. *CDC/NIOSH/HELD, Morgantown, WV.*

Single-wall carbon nanotubes (SWCNT) are of great interest to many aspects of industry including, but not limited to, electronics and pharmaceutics. As SWCNT-manufactured goods are being introduced into market, concerns regarding their potential for producing environmental and human health risks have been raised. In the present study, we investigated SWCNT effects on the human broncho-epithelial cells (BEAS-2B. Exposure of BEAS-2B cells to SWCNT resulted in dose-dependent loss of cell viability and oxidative stress by means of dose-dependent increase in OH radical production and superoxide dismutase activity (SOD). Furthermore, SWCNT induced time- and dose-dependent apoptosis and phosphorylation of histone γ-H2A.X, a variant of histone H2A that is activated following DNA damage. Whether SWCNT have the potential to induce apoptosis, BEAS-2B cells were exposed for different time points to SWCNT (50 μg/cm2), and analyzed for PARP activation, as a molecular marker of apoptosis. The level of cleaved PARP increased after 18h of exposure. This level decreased when cells were allowed to recover for 6h in normal growth media. We also examined the ability of SWCNT to induce the transactivation of activator protein-1 (AP-1) and nuclear factor-kappaB (NF-κB), transcription factors that are important members of signal transduction pathways. SWCNT induced greater activation of AP-1 and NF-κB at lower doses as compared to higher doses. These effects were diminished by PD98059, an inhibitor of MAP kinase. Crocidolite asbestos was used as a positive control along with SWCNT, and the effects of crocidolite were far greater as compared to SWCNT. Considering the role of airway epithelium as a barrier and its role in pulmonary functioning, the results of the present study suggest that interaction of SWCNT with airway cells may cause adverse biological responses that may initiate respiratory diseases.
Key words: Apoptosis, asbestos, carbon nano-particles, cell toxicity, oxidative stress,

PS 2214 AEROSOLIZATION DELIVERY SYSTEM OF AIRBORNE NANOPARTICLES FOR NOSE-ONLY INHALATION STUDIES.

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Understanding human health risks associated with engineered nanomaterials is particularly challenging because of the wide range of plausible exposure scenarios. While workers, consumers, or the general public may potentially be exposed to nanoparticles through a number of pathways (e.g., dermal, ingestion, ocular), inhalation, at least from an occupational standpoint, is likely to be one of the most significant routes of exposure. For hazard assessment of inhaled nanoparticles, it is critical to have a means to deliver respirable airborne nanoparticles for experimental animal studies. An aerosolization system was developed to administer nanomaterials from a dry bulk media into respirable airborne particles for delivery into a nose-only inhalation system. Utilization of a cannula-based feed system, diamond grinding wheel, cyclone-type conditioning chamber, and Krypton-85 source (charge neutralization) allows for efficient delivery of otherwise difficult to produce respirable-size particles. Different nanomaterials (e.g., single-walled carbon nanotubes, ultrafine carbon black) were tested with the aerosolization system and aerosolized particles were characterized by size, mass, and number distribution using a gravimetric filter analysis, inertial cascade impactor, and scanning mobility particle sizer with a condensation particle counter, as well as by particle morphology using transmission electron microscopy. Aerosolized particles represented a wide range of size and morphological characteristics with particles spanning the fine (0.1-2.5 μm) and ultrafine (<0.1 μm) size range mostly in an agglomerated state. An advantage that this system offers over other aerosol-generating systems is that it utilizes relatively small amounts of dry material (<0.3 g) to generate respirable particle concentrations up to 1 mg/m3 continuously over a 6-hr period. Relating exposure characteristics of airborne particles in experimental studies to those in human exposure settings will be important for establishing exposure/dose-response relationships and standards to protect human health.

PS 2215 COMPARISON OF CARBON NANOTUBE-INDUCED CYTOTOXICITY IN A549 AND NORMAL HUMAN BRONCHIAL EPITHELIAL (NHBE) CELLS.

V. Walker, T. Hulderman and P. P. Simeonova. *Health Effects Laboratory Division, NIOSH/CDC, Morgantown, WV.*

The most attractive features of nanomaterials including their small size, large surface area, and reactivity might also be the main factors for their toxicity. Pulmonary bronchial epithelial cells are a potential target for toxicity during respiratory exposure. The tumor cell line A549, a pulmonary human type-II like epithelial cells, is

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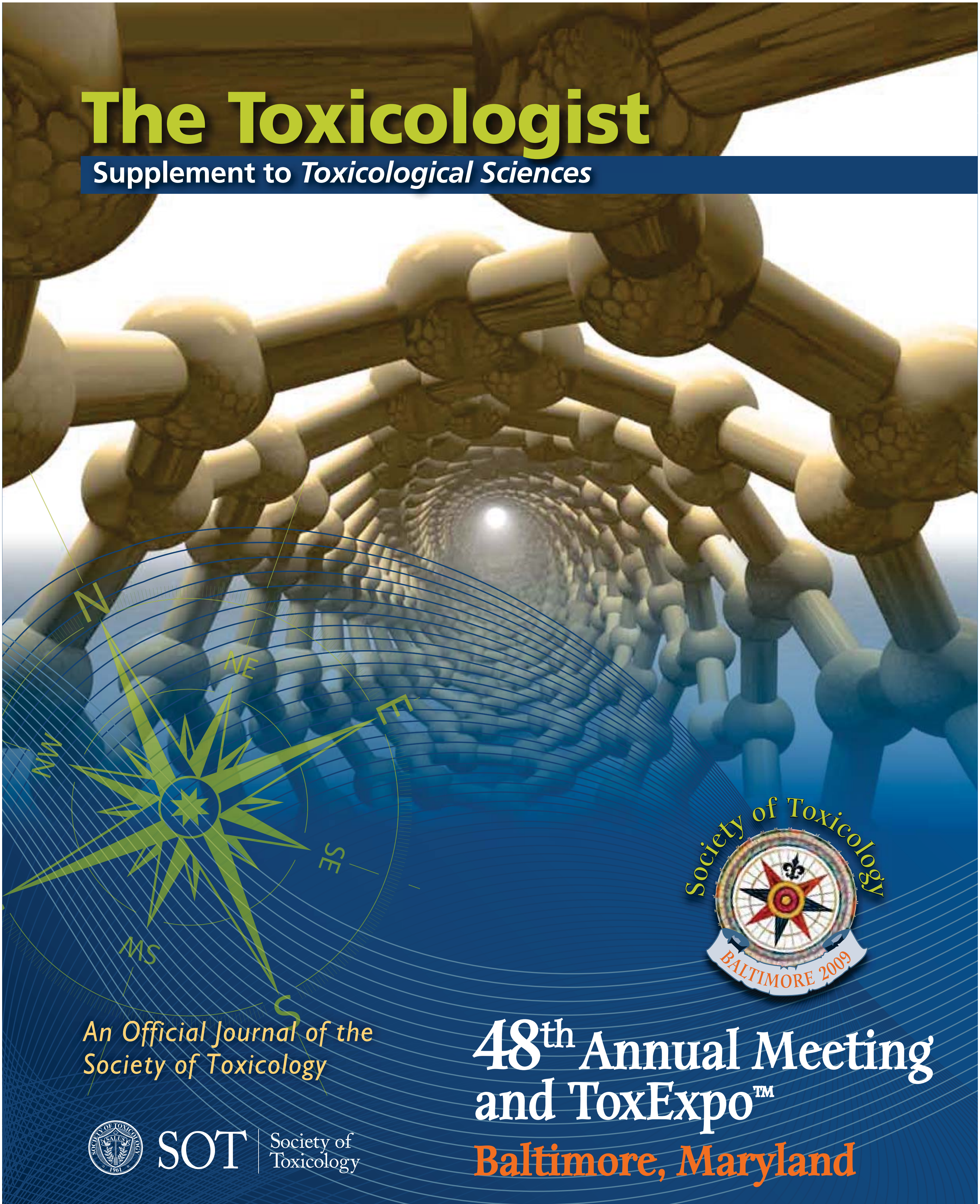

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Deadline for Proposals for SOT 2010 Annual Meeting Sessions: April 30, 2009

WHY SUBMIT A PROPOSAL?

1. To present new developments in toxicology.
2. To provide attendees an opportunity to learn about state-of-the-art technology and how it applies to toxicological research.
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Note: CE Courses will be held on Sunday.

Symposia—"Cutting-edge" science; new areas, concepts, or data

Workshops—State-of-the-art knowledge in toxicology

Roundtables—Controversial subjects

Historical Highlights—Review of a historical body of science that has impacted toxicology

Informational Sessions—Scientific planning or membership development

Education-Career Development Sessions—Sessions that provide the tools and resources to toxicologists that will enhance their professional and scientific development

2010 Thematic Approach

The Scientific Program Committee will continue the thematic approach for the 2010 Annual Meeting. All proposal submissions will be reviewed for their relevance under the following themes—***Cell Signaling, Gene-Environment Interactions, Metabolic Disease, Mitochondrial Basis of Disease, Toxicity Testing in the 21st Century,*** and ***Translational Toxicology*** for the 2010 meeting. Please note that while we are actively soliciting proposals for the themes listed above, all proposal submissions will be reviewed under the current criteria for their timeliness and relevance to the field of toxicology.

Please refer to the SOT 2009 *Program*, Scientific Program Overview on the fold-out cover for a list of 2009 sessions highlighted under the thematic approach.

You can now submit your proposal on-line at www.toxicology.org

Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the continuing education, symposia, workshop, roundtable, platform, and poster discussion sessions of the 48th Annual Meeting of the Society of Toxicology, held at the Baltimore Convention Center, March 15–19, 2009.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 469.

The issue also contains a Key Word Index (by subject or chemical) of all the presentations, beginning on page 487.

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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