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

widely used for evaluation of particle toxicity. We hypothesized that A549 cells are more resistant to toxicity compared to the normal epithelial cells. To test this hypothesis we examined the dose-dependent cytotoxicity effects of single-walled (SWCNT) and multi-walled (MWCNT) carbon nanotubes on NHBE and A549 cells using lactate dehydrogenase (LDH) release and the cell proliferation/viability WST-1 assays. We found that NHBE exhibited marked cytotoxicity, similar to that observed with hydrogen peroxide treatment (positive control), consistently by 24 hours post-exposure to high concentrations [50-150μg/106 cells (1.5-4.5μg/ml)] of CNT. In comparison, exposure of A549 cells to similar concentration of CNT resulted in only a milder response. Overall, the results presented here indicate that SWCNT or MWCNT induce cytotoxicity in human lung epithelial cells in a time and dose-dependent manner. Furthermore, we demonstrate a large variation between the particle-induced cytotoxic responses in A549 cells and normal primary epithelial cells.

PS 2216 CELLULAR AND MOLECULAR MECHANISMS OF BROMATE-INDUCED TOXICITY IN RAT AND HUMAN KIDNEY CELLS.

X. Zhang[1], R. J. Bull[2], J. A. Cotruvo[3], J. Fisher[4] and B. S. Cummings[1].
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The cellular and molecular mechanisms of bromate-induced toxicity in normal rat kidney (NRK) and human embryonic kidney cell lines (HEK293) after acute exposures were investigated. Bromate (added as $KBrO_3$ at 0-0.8 mg/ml) exposure decreased MTT staining after 48 hr in both cell lines. Bromate also increased phosphatidylserine externalization in tandem with decreases in membrane integrity after 48 hr as assessed by annexin V and PI staining. Cell cycle analysis demonstrated that bromate induced G2/M arrests in both cell lines. These data suggest that the mechanism of cell death induced by bromate is necrosis. Immunoblot analysis demonstrated that G2/M arrest correlated to induction of p53, p21 and phospho-cdc2 in HEK293 cells. Further, bromate treatment induced both time- and concentration-dependent increases in the activity of the mitogen activated proteins kinases (MAPK) p38 and ERK1/2. The activity of p38 and ERK1/2 increased after just 15 min of bromate exposure, prior to any increase in cell death or cell cycle arrest. Inhibition of p38 and ERK1/2 using SB202190 and PD98059, respectively increased bromate-induced decreases in MTT staining. Interestingly, treatment of cells with SB202190, but not PD98059, partially reversed bromate-induced G2/M arrest in HEK293 cells. Further, bromate exposure increased the expression of thioredoxin 1 in HEK293 cells after just 15 min. These data demonstrated the novel finding that bromate induced activation of MAPK and thioredoxin, which correlates to cell cycle arrest and cell death. This work was supported by Awwa RF 4042, IOA, MWD, NWRI, Calleagas Water, Long Beach Water, SNWA, LADWP, Veolia, Environment Abu Dhabi and the Georgia Cancer Coalition.

PS 2217 THE NOVEL TUMOR PROMOTER PALYTOXIN ACTIVATES EXTRACELLULAR SIGNAL REGULATED KINASE 5 THOUGH A NA+, K+-ATPASE-DEPENDENT PATHWAY.

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Palytoxin is classified as a non-12-O-tetradecanoylphorbol-13-acetate (TPA)-type skin tumor because it does not bind to or activate protein kinase C. The novel characteristics of palytoxin led us to investigate how it affects cellular signal transduction pathways. We previously showed that palytoxin stimulates the activation of three major members of the mitogen activated protein kinase (MAPK) family, c-Jun N-terminal kinase (JNK), p38, and extracellular signal regulated kinase (ERK) 1/2. Here we report palytoxin also activates ERK5, another MAPK family member, in mouse keratinocytes derived from initiated mouse skin (308 cells) and HeLa cells. By contrast, TPA does not activate ERK5 in these cell lines. The putative cell surface receptor for palytoxin is the Na+, K+-ATPase. Accordingly, ouabain blocked the ability of palytoxin to activate ERK5. We did not detect the activation of ERK5 by ouabain alone, however, indicating a divergence in the signaling pathways activated by these two ligands for the Na+, K+-ATPase. The activation of ERK5 by palytoxin was not mimicked by cycloheximide, okadaic acid, or sodium orthovandate. These results indicate that the stimulation of ERK5 by palytoxin is not simply due to its ability to inhibit protein synthesis or to inhibit tyrosine or serine/threonine phosphatases. Thus palytoxin appears to activate ERK5 through a mechanism that differs significantly from the mechanisms by which it activates JNK, p38, and ERK1/2. The observation that ERK5 is involved in the regulation of cell proliferation and survival suggests that ERK5 may be an important target of palytoxin action.

PS 2218 INHIBITION OF CALCIUM-INDEPENDENT PHOSPHOLIPASE A_2 ACTIVATES MAP KINASE SIGNALING PATHWAYS DURING CYTOTOXICITY IN PROSTATE CANCER CELLS.

B. Sun, X. Zhang and B. Cummings. *Pharmaceutical and Biomedical Sciences, UGA, Athens, GA.*

The p53-dependent and -independent signaling pathways activated during cytotoxicity induced by Ca^{2+}-independent phospholipase A_2 ($iPLA_2$) inhibitors in prostate cancer cells were investigated. $iPLA_2$ inhibition using siRNA, or the selective inhibitor bromoenol lactone (BEL), decreased growth in LNCaP (p53 positive) and PC-3 (p53 negative) human prostate cancer cells. Decreased cell growth correlated to time- and concentration-dependent activation of the mitogen activated protein kinases p38 and ERK1/2. p38 was activated in both cell lines, while ERK1/2 was transiently activated in only PC-3 cells. Inhibition of p38 using SB202190 inhibited the ability of BEL to activate p53 and p21 in LNCaP cells, and reversed BEL-induced G2/M arrest in PC-3 cells. In contrast, inhibition of ERK1/2 using PD98059 only slightly altered p53 activation in LNCaP cells. Interestingly, $iPLA_2$ inhibition significantly increased the activity of epidermal growth factor receptors (EGFR) in PC-3 cells, but not LNCaP cells. Treatment of PC-3 cells with the EGFR inhibitor AG1478 prevented BEL-induced ERK1/2 activation. Similar results were seen using the matrix metalloproteinases inhibitor GM6001. Neither of these inhibitors altered BEL-induced p38 activation. Inhibition of ERK1/2 prior to treatment with BEL decreased PC-3 cell growth in comparison to cells exposed to BEL alone. Similar results were seen in LNCaP cells when p38 was inhibited. In contrast, inhibition of p38 in PC-3 cells protected against BEL-induced cytostasis. These data demonstrate the novel findings that $iPLA_2$ inhibition differentially activates p38 and ERK1/2 in prostate cancer cells, and further suggest that these signaling kinases have differential roles in prostate cancer cell growth.

PS 2219 THE TUMOR SUPPRESSOR GENE TSC-2 MODULATES TRANSLATION INITIATION OF CYCLIN D1 THROUGH ERK CROSSTALK WITH 4EBP1.

J. D. Cohen[1], J. M. Gard[2], R. B. Nagle[2], T. J. Monks[1] and S. S. Lau[1].
[1]Pharmacology and Toxicology, University of Arizona, Tucson, AZ and [2]AZCC, U of A, Tucson, AZ.

The mTOR and MAPK signaling cascades have been implicated in a number of human cancers. The tumor suppressor gene tuberous sclerosis-2 (Tsc-2) functions as a negative regulator of mTOR. Critical proteins in both pathways are activated following treatment of Eker rats (Tsc-$2^{EK/+}$) with 2,3,5-tris-(glutathion-S-yl) hydroquinone (TGHQ), which also results in loss of the wild-type allele of Tsc-2 in renal preneoplastic lesions and tumors. Western blot analysis of kidney tumors formed following 8-months of TGHQ treatment of Tsc-$2^{EK/+}$ rats revealed increases in B-Raf, C-Raf, p-ERK, cyclin D1, 4EBP1, and p-4EBP1-Thr70, -Ser65, and -Thr37/46 expression. Concomitant with increases in expression of these proteins in TGHQ-induced tumors, similar changes are observed following TGHQ transformation of primary renal epithelial cells derived from Tsc-$2^{EK/+}$ rats (QTRRE cells). QTRRE cells are null for tuberin due to loss of heterozygosity at the Tsc-2 allele and exhibit high ERK, B-Raf and C-Raf kinase activity, and increased expression of cyclin D1, 4EBP1, and all p-4EBP1s. Following siRNA knockdown of C-Raf, Western blot analysis revealed a significant decrease in C-Raf, cyclin D1, and all p-4EBP1 forms noted above. In contrast, siRNA knockdown of B-Raf resulted in a nominal change in these proteins. Furthermore, treatment of the QTRRE cells with the Raf kinase inhibitor Sorafenib (therapeutic anti-cancer agent) and the MEK1/2 kinase inhibitor PD 98059, both produced a significant decrease in the protein expression of cyclin D1 and all p-4EBP1s. Western analyses revealed three distinct species of 4EBP1, with the lowest molecular weight band lacking phosphorylations on Thr65 and Thr70. Moreover, treatment with both inhibitors causes an increase in the relative abundance of all three bands. The data indicate that Raf-MEK-ERK participates in crosstalk with 4EBP1, which represents a novel pathway interaction leading to increased protein synthesis, cell growth, and kidney tumor formation. (GM39338, ES007091, ES06694)

PS 2220 OVERLAPPING SIGNAL SEQUENCES AND PHOSPHORYLATION CONTROL NUCLEAR LOCALIZATION AND ENDOPLASMIC RETICULUM RETENTION OF GLUCOSE REGULATORY PROTEIN GRP58.

E. Unni, A. K. Adikesavan and A. K. Jaiswal. *Pharmacology and Exp. Therapeutics, Univ of Maryland School of Medicine, Baltimore, MD.* Sponsor: B. Moorthy.

Glucose regulatory protein (GRP58), has two thioredoxin-like domains and is known to function as thiol-dependent oxidoreductase. GRP58 is present in cytosol, nucleus and endoplasmic reticulum (ER). GRP58, retained in ER, specifically in-

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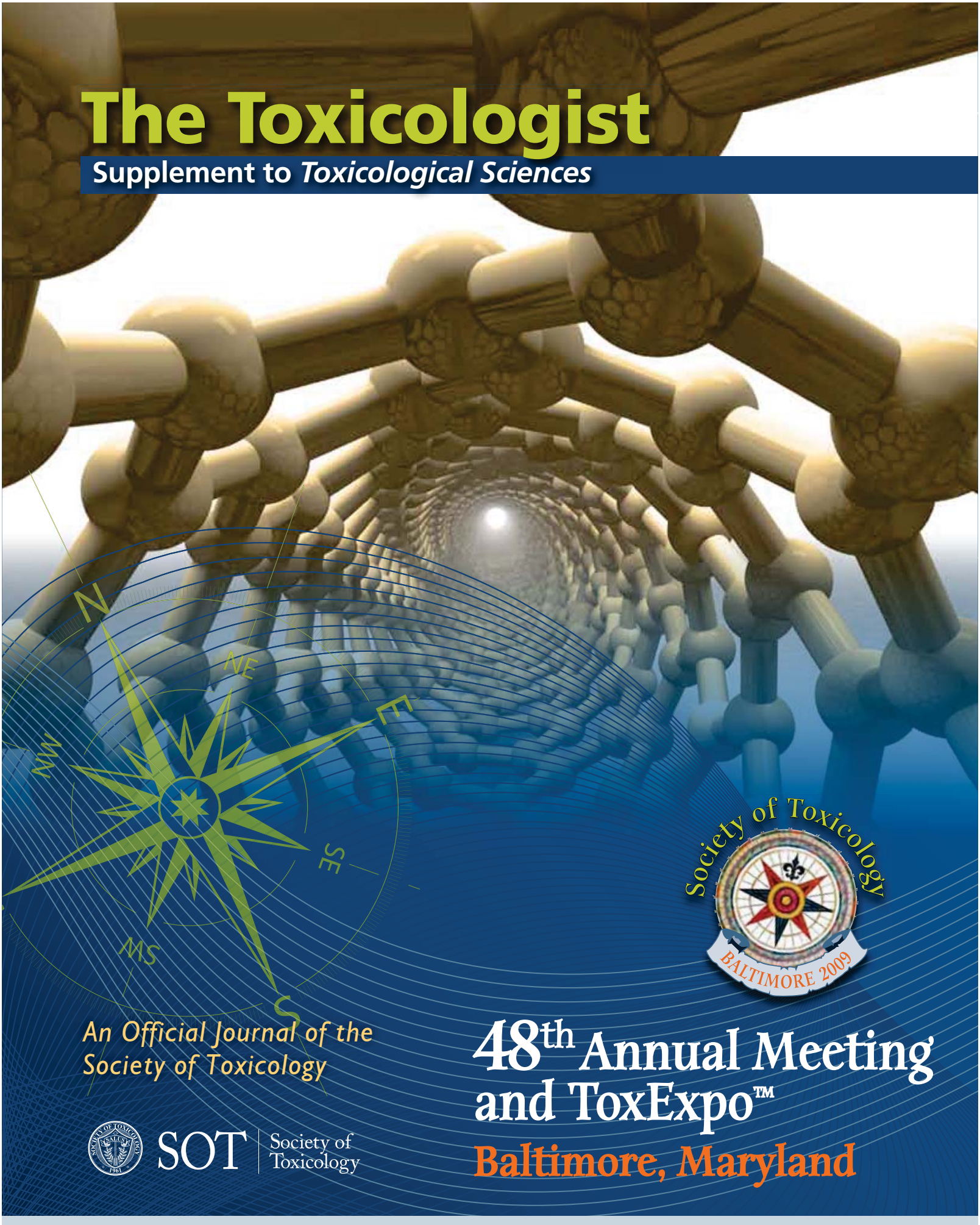

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2010 Thematic Approach

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.

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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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