PS 2077 EVALUATION OF CORTICOSTERONE AND METABOLITES IN A CHRONIC MILD STRESS RAT MODEL.

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Corticosterone is a steroid hormone secreted by the adrenal gland and is involved in the regulation of fuel metabolism, immune reactions, and stress responses. When clinical signs of stress and changes to immune organs are observed during drug development studies, differentiating between direct immunotoxicity and changes mediated by stress can be difficult. Recent research suggests that when corticosterone levels are elevated by either administration of exogenous corticosterone or by exposure to environmental stressors, changes in tissues known to be affected by stress resulted. The measurement of corticosterone during drug development studies may allow researchers to predict if changes in immune organs are the indirect result of stress or a direct drug related effect on the immune system. Previously, assessment of corticosterone levels required multiple blood samples collected over a period of time. Serial blood sampling is unfeasible in small animals, and the use of urinary corticosterone is becoming the preferred method for corticosterone assessment. A correlation between blood and urinary corticosterone has been observed and studies are ongoing to assess the use of urinary corticosterone as a pre-clinical biomarker of stress in rats (J. Immunotoxicol, 4:25-38, 2006). To aid in biomarker development, an assay to measure corticosterone and two metabolites in female rat urine has been developed. The assay utilizes HPLC coupled with mass spectrometric detection. The linear range is 0.05 to 2.0 µg/ml and assay precision is within 22%. The assay was used to measure corticosterone and two metabolites in the urine of rats exposed to chronic mild stress with and without treatment with Imiprimine. Higher levels of corticosterone and 6-α hydroxycorticosterone were observed in the urine of rats exposed to chronic mild stress without Imiprimine treatment when compared to the vehicle control rats. Corticosterone sulphate levels were below the LLOQ for most of study samples but future plans include concentrating or extracting the urine to enhance the measurement of the sulphate metabolite.

PS 2078 THE DETECTION OF NK CELLS FOLLOWING THE ADMINISTRATION OF BIOENGINEERED MONOCLONAL ANTIBODIES TO NON-HUMAN PRIMATES.

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CD56 is the most commonly used antigen for immunophenotypic analysis of Natural Killer (NK) cells in humans; however this antigen is not consistently present on non-human primate (NHP) NK cells, a key animal model used in preclinical safety assessment and immunotoxicology studies of biopharmaceuticals in development. The antigen most often used to identify NK cells in the NHP is CD16 (FcγRIIIA, the low affinity Fc receptor). Recently the efficacy of many new antibody biopharamaceuticals has been enhanced through engineering of the Fc fragment to increase antibody dependent cell-mediated cytotoxicity (ADCC). However, the administration of an antibody biopharmaceutical with an engineered Fc fragment often interferes with immunophenotypic detection of the CD16 antigen in NHPs, making it appear as though either CD16 expression or the total NK population has been reduced. To characterize the apparent alterations in NK cells associated with CD16 labeling, CD159a (NKG2A which does not bind Fc fragments) was evaluated as a second NK cell marker in NHPs. CD159a was observed to bind approximately 80% of the cells that label positive with CD16 in cynomolgus monkeys. Because of the potential for bioengineered monoclonal antibodies to alter the ability to detect NK cells by immunophenotyping, both CD16 and CD159a were evaluated in several NHP preclinical studies. In one study, cynomolgus monkeys were administered an antibody biopharmaceutical with an engineered Fc fragment once weekly for 5 weeks. Through the dosing phase of the study there was a reduction in CD16 labeling of NK cells; with a corresponding albeit much smaller reduction in CD159a+ NK cells, consistent with a true antibody-associated decrease in cell numbers, but to a much smaller magnitude than suggested by the anti-CD16 data. In this and additional evaluations with antibody biopharmaceuticals, the ability to evaluate alterations in NHP NK cells has been enhanced by the utilization of CD159a in conjunction with CD16 to monitor the anti-CD16/FcγRIIIA (CD16) receptor dynamics.

PS 2079 AMIODARONE INDUCES PRO-FIBROTIC AGENTS IN RAT PLEURAL MESOTHELIAL CELLS.

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Clinical studies have demonstrated an association between amiodarone therapy and a variety of pulmonary complications ranging from sub-acute necrotizing pneumonitis to pulmonary fibrosis. Fibrosis is characterized by increased expression of extracellular matrix proteins and such expression may involve pro-fibrotic agents such as Angiotensin II (Ang II), Transforming Growth Factor β1 (TGF β1) and Connective Tissue Growth Factor (CTGF). This study investigated the effect of amiodarone on the levels of the pro-fibrotic proteins TGF β1, CTGF and Ang II and the extracellular matrix protein elastin in rat pleural mesothelial cells (RPMCs). RPMCs were maintained in Ham's F-12 medium at 37°C in a humidified atmosphere of 5% CO2 / balanced air. Confluent cultures of RPMCs were treated with 5µg/ml of amiodarone for 24hrs, followed by protein extraction and western blot analyses for CTGF and TGF β1. To investigate the effect of amiodarone on elastin, confluent cultures were treated with 5µg/ml of amiodarone for 24hrs, after which the original medium was replaced with medium containing 100µg/ml of β-aminopropionitrile to prevent elastin cross-linking. Effects of amiodarone on Ang II levels were studied by treating RPMCs with 5µg/ml of amiodarone for 24hrs. After 5 days of additional culture, levels of soluble elastin and Ang II present in the medium were analyzed using Enzyme Linked Immunosorbent Assays. Amiodarone treated cultures had increased levels of TGF β1 (132%±5.8; $P<0.05$) and CTGF (202.2%±9.7; $P<0.05$) in comparison to controls. Perturbation of RPMCs by amiodarone increased the accumulation of soluble elastin (197.8%±17.2; $P<0.001$) and Ang II when compared to controls. Such findings suggest that amiodarone induces the expression of pro-fibrotic proteins that in turn increase levels of extracellular matrix proteins creating a fibrotic-like response in RPMCs.

PS 2080 EFFECT OF PARTICLE SIZE ON THE REGIONAL DEPOSITION OF TECHNITIUM-99M LABELED PARTICLES IN RODENTS.

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There is extensive information detailing the deposition patterns of various particles in the human respiratory tracts and several manuscripts detailing deposition in preclinical species. However, the majority of the data in rodents was generated prior to significant advancements in rodent imaging capabilities. In order to address this need and determine the effect particle size has on the deposition patterns in rodent species these experiments were conducted. Rats and mice were exposed, via nose only inhalation, to particles ranging from 0.5 to 5 microns mass median aerodynamic diameter (MMAD). The aerosols were composed of technetium-99m radiolabeled sulfur colloid particles. Aerosols were generated with a series of compressed air jet nebulizers to achieve each desired particle size (0.5, 1.0, 3.0 and 5.0 micron). Aerosol samples were collected to characterize the activity aerosol concentration and the particle size distribution. Impactor analysis detailed that mass and activity median aerodynamic diameters (AMAD) correlated with each other. For example for the target particle size of 0.5 micron the MMAD was 0.62 micron and the AMAD was 0.57 micron. These data indicate that the aerosols of Tech-99m and the sulfur colloid particles were homogeneous. This trend of MMAD and AMAD agreement was consistent from 0.5 to 5 microns. In order to determine deposition animals were sacrificed immediately following inhalation exposures and were imaged using a small-animal SPECT-CT camera. Data analysis indicated that 11 µCi of material in lungs of the mouse and 60 µCi in the lungs of the rat after exposure to the 0.5 micron aerosol. Increasing particle size resulted in an increase in deposition in the nasal region and resulted in an increase in stomach uptake.

PS 2081 ADHESION MOLECULE EXPRESSION AND CYTOTOXICITY IN DIACETYL EXPOSED RAT LUNGS.

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Diacetyl (2,3-butanedione), a volatile component of butter and butter flavoring, gives food the flavor and aroma of butter, and has been associated with fixed airways obstruction in the microwave-popcorn and flavoring industries. Inhaled diacetyl vapors cause epithelial necrosis in the rat and mouse upper respiratory tract. Many cytotoxic agents cause both apoptotic and necrotic cell death but the role of apoptosis in diacetyl-induced cell death is unknown. Dicarbonyl/xylulose reductase (DCXR) metabolizes diacetyl to acetoin and appears to play a role in mucosal absorption of diacetyl. In human skin, DCXR localizes to the cytoplasm near the adhesion molecules, e-cadherin and β-catenin. Therefore, we hypothesized that diacetyl causes apoptosis and changes in epithelial adhesion molecules. Lungs were collected from Sprague Dawley rats one day after a 6 h exposure to inhaled air (n=6) or 317 ppm diacetyl vapor (n=6). Apoptosis was assessed by the TUNEL assay and immunofluorescence for activated caspase-3. DCXR, e-cadherin, and β-catenin were visualized by immunofluorescence and confocal microscopy. Diacetyl

increased the number of TUNEL and activated caspase 3 positive cells in mainstem bronchus epithelium. However, most cells in necrotic foci did not exhibit these apoptotic markers. DCXR staining was most intense in terminal bronchiolar cells morphologically consistent with Clara cells. In some necrotic foci in the mainstem bronchus epithelium, the normal thin linear staining of β-catenin at intercellular junctions was replaced by more focal globular expression. Detaching necrotic cells also often lost β-catenin and e-cadherin expression. Such alterations may play a role in, or possibly result from, necrosis, apoptosis, and/or epithelial detachment. These findings indicate that both apoptosis and necrosis contribute to diacetyl-induced epithelial injury and suggest that diacetyl may alter intercellular adhesion complexes of respiratory epithelium.

PS 2082 REPEATED EXPOSURE OF HUMAN BRONCHIAL EPITHELIAL CELLS TO OZONE SUPPRESSES THEIR RESPONSE TO STAPHYLOCOCCUS AUREUS.

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Ozone (O3) is a common air pollutant that causes a variety of adverse health effects including altered host vulnerability to pathogens. Pulmonary clearance of Staphylococcus aureus is decreased after O3 exposure, but the mechanisms that underlie altered host susceptibility are poorly understood. Our goals were to determine whether UNCN3T cells, a human bronchial epithelial cell line grown at air-liquid interface, secrete pro-inflammatory cytokines in response to repeated exposure to environmentally relevant levels of O3, and if repeated O3 exposures alter responsiveness of these cells to a S. aureus filtrate challenge. We determined that this cell line did not release cytokines following exposure to O3. We found that repeated exposures to a low dose (0.2 ppm) and high dose (0.8 ppm) of O3 followed by a S. aureus filtrate challenge attenuated the release of MIP-3α, Gro-α, IL-6, ENA-78, and G-CSF, as compared to air control cells. Furthermore, O3 pre-exposure significantly decreased phosphorylation of p65 20 minutes and 60 minutes after administration of the bacteria filtrate and significantly decreased phosphorylation of ERK1/2 20 minutes after the S. aureus filtrate challenge. These results indicate that repeated exposures to low level O3 attenuates airway epithelial pro-inflammatory responses to S. aureus filtrate This response could increase host susceptibility to this prevalent pathogen.

PS 2083 OZONE-INDUCED EXACERBATION OF ACUTE LIVER INJURY IN MICE.

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Ozone (O3), an oxidant air pollutant in photochemical smog, principally targets epithelial cells lining the respiratory tract. However, changes in global gene expression have also been reported in livers of O3-exposed mice. Overdose with acetaminophen (APAP) is the most common cause of drug-induced liver injury in developed countries. In the present study, we examined the effects of a single, ambient O3 exposure on livers of mice treated with a hepatotoxic dose of APAP. C57BL/6J male mice were fasted overnight and then given APAP (300 mg/kg ip) or saline vehicle (0 mg/kg APAP). Two hours later, mice were exposed to 0 or 0.5 ppm O3 for 6 hours. They were sacrificed 1 or 24 hours after O3 exposure. Animals killed at the 24-hour timepoint were given bromodeoxyuridine (BrdU) two hours before sacrifice to identify hepatocytes undergoing reparative DNA synthesis. Liver tissue samples were processed for light microscopic examination and morphometric analyses. Blood samples were analyzed for plasma alanine aminotransferase activity (ALT). Saline-treated mice exposed to either air or ozone had no liver injury. All APAP-treated mice developed marked hepatic centrilobular necrosis that increased in severity with time after APAP exposure. O3 exposure increased the severity of APAP-induced liver injury as indicated by a 60% and 33% increase in necrotic hepatic tissue and plasma ALT, respectively. APAP also induced a 10-fold increase in BrdU-labeled hepatocytes that was 80% attenuated by O3. Gene expression analysis at 1 hour after O3 revealed that APAP and O3 coexposure resulted in greater expression of p21 and Socs3 mRNA and less IL-6 mRNA expression compared to APAP alone. These results suggest that acute exposure to oxidant air pollution exacerbates drug-induced liver injury and delays hepatic repair.

PS 2084 INHALED GLUCOCORTICOID CAUSES RAPID VASOCONSTRICTION IN THE ISOLATED AND PERFUSED RAT LUNG.

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Rationale: Recently it was shown in the clinic that inhaled corticosteroids cause rapid bronchial vasoconstriction. The aim of the present study was to investigate whether vasoconstrictive effects on pulmonary circulation could be detected after short inhalation of a glucocorticoid in a preclinical model: the isolated and perfused rat lung (IPL). Methods: Using the recently developed DustGun Technology, we exposed the IPL by inhalation to a dry powder aerosol of budesonide (BUD). The pulmonary perfusate flow rate was assessed during 100 min post dose. A reduction in perfusion flow was interpreted as vasoconstriction. Main Results: The onset of vasoconstriction was significant within 10-40 min after inhalation exposures to 10 and 50 μg BUD compared to vehicle (lactose) (p=0.012 and 0.049, respectively). Throughout the whole perfusion period (100 min), 2 μg BUD had no effect while 10 μg had more pronounced effect than 50 μg BUD. Co-administration of a selective α1-adrenoceptor antagonist (prazosin 50 nM added to the perfusate) with 10 μg BUD reduced vasoconstriction by approximately 50% during 100 min of perfusion (p=0.003).
Conclusions: The results suggest that corticosteroid-induced airway vasoconstriction is at least partly driven by an α1-adrenoceptor mediated mechanism. The rapid onset of vasoconstriction after inhalation of budesonide suggests that this is a nongenomic effect. This ex-vivo study supports clinical observations of vasoconstriction after inhalation of corticosteroids. This model could serve as a complement to clinical models to uncover effects and mechanisms of pulmonary/bronchial circulatory changes.

PS 2085 IMMUNE RESPONSES IN NEONATAL AND ADULT RATS EXPOSED TO SOOT.

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Exposure to particulate air pollution (PM) has been correlated with increased morbidity and mortality in adults and with increased asthma in children. PM contains combustion products including organic compounds bound to particulates and in the gas phase. We exposed neonatal (7d old) and adult male Sprague Dawley rats to inhaled soot generated by diffusion flame for 6 hrs. Control rats were exposed to filtered air. This soot was low in organic carbon; simulating the particulate fraction of PM. Airway epithelial cell injury was assessed 2 hrs post exposure using differential permeability to fluorescent dyes, high resolution histopathology and by measuring LDH release into bronchoalveolar lavage fluid (BALF). BALF and peripheral blood differentials as well as cytokine profiles in serum and BALF were measured at 6, 24 and 48 hours post PM. We found that exposure to soot stimulated an increase in circulating neutrophils in adult rats and an increase in lavage neutrophils in neonates. LDH release was significantly increased in the BALF of neonates only at the 2 hr timepoint but cellular injury was not apparent at either age. Cytokines measured by a multiplex assay in both BALF and the peripheral blood were altered by exposure to PM compared to age matched filtered air controls. There were temporal differences by age. BALF IL-1Beta significantly increased at 2 hrs following PM in adult rats. TNF-a ($P<0.0092$) and IL-6 ($P<0.0211$) cytokine levels in serum were decreased in neonates 48 hrs post PM. We conclude that low PAH soot does not cause frank cytotoxicity in airways of neonatal or adult rats but that exposure to soot causes changes in neutrophil abundance and changes in key cytokines in both the lavage and peripheral blood. There are temporal and spatial differences in neonatal responses compared to adult responses. Funded by EPA RD83241401 and NIH ES/HL06700. Although funded in part by the USEPA this research has not been subject to USEPA required peer and policy review and therefore does not reflect the views of the Agency and no official endorsement should be inferred.

PS 2086 SYSTEMIC DISPOSITION OF INHALED NITRIC OXIDE, A SIGNIFICANT COMPONENT OF VEHICULAR EMISSIONS.

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Nitric oxide (NO) is a common component of fresh diesel and gasoline engine emissions that rapidly transforms both in the atmosphere and once inhaled. Because of this rapid transformation, extremely limited information is available in terms of potential human exposures and health effects. Healthy young rats were exposed to whole diesel emissions (DE) adjusted to 300 $\mu g/m^3$ of particulate matter, filtered air (FA), or 10 ppm NO as a positive control. Animals exposed to DE and

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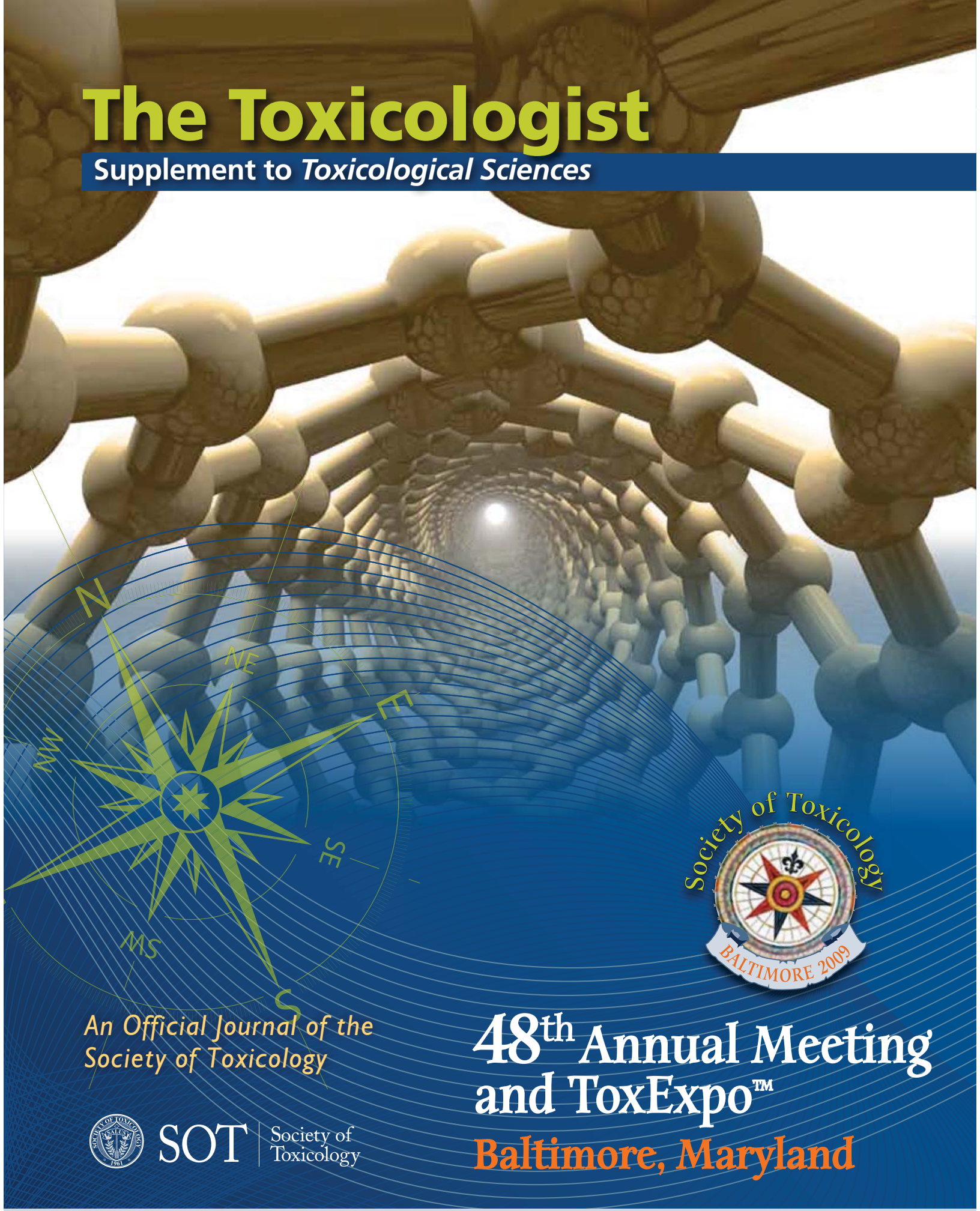

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