PS 246 GOLD NANOPARTICLE (AUNP) SURFACE CHARGE INFLUENCES TOXICITY.

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Nanoparticles can vary in size, chemical composition, surface chemistry and surface area. Small changes in these features impart different properties to a nanoparticle, including how they interact with and potentially influence biology. Early life stages are often sensitive to chemical insult, which make embryonic zebrafish as an ideal platform to investigate the biological activity and toxic potential of gold nanoparticles (AuNP). In initial studies, developmental responses to waterborne exposure to AuNPs were determined using a wide range of concentrations (0.016 to 250 µg/mL (ppm)). Cationic, N,N,N trimethylammoniumethanethiol (TMAT), and anionic, 2-mercaptoethanesulfonate (MES), AuNPs produced with a 1.5nm core size induced developmental toxicity in embryonic zebrafish. TMAT was more overtly developmentally toxic, while MES induced more sublethal effects at this developmental stage. The most susceptible early developmental stage to nanomaterial exposure was determined to be 24 hours post fertilization (hpf) and 48 hpf for TMAT and MES, respectively. Narrowing the window of exposure allowed for genomic evaluations to be conducted in order to determine gene expression responses to each AuNP exposure. Nimblegen zebrafish arrays with 37,157 genes were used with RNA isolated from embryonic zebrafish exposed to the lowest observable adverse effect (LOAEL) for TMAT (50ppm) and MES (10 ppm) at 24 and 48 hpf. The combination of these studies illustrates the value of the embryonic zebrafish model to determine structure activity relationship, and that the surface charge of AuNP influences biological responses. Supported by NIEHS P3000210, EPA RD-833320, T32 ES07060, and Air Force Research Laboratory under agreement # FA8650-05-1-5041.

PS 247 PULMONARY BIOASSAY STUDIES WITH SEPIOLITE NANOCLAY SAMPLES IN RATS.

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Sepiolite is a magnesium silicate nanoclay mineral. A pulmonary bioassay toxicity study was conducted in male rats with sepiolite, PBS, quartz particles and ultrafine titanium dioxide particles. Following intratracheal instillation exposures at doses of 1 and 5 mg/kg, the groups were evaluated by bronchoalveolar lavage (BAL) at 24 hours, 1 week, 5 weeks, and 3 months postexposure. Results showed that quartz particles produced sustained, lung inflammation. Exposures to uf TiO2 particles, sepiolite or PBS produced transient lung inflammation. However, unlike the other particle-types, sepiolite clay exposures produced macrophage-agglomerates or giant cells, recovered in BAL fluids at 1 wk, 5 wk and 3 month postexposure time periods. The biological significance of these findings is unknown. Following the bronchoalveolar lavage procedure, the lungs of randomly selected rats were then infused with formaldehyde fixative. Lung tissues from PBS control, high-dose Min-U-Sil quartz, negative control ultrafine TiO2, low and high dose sepiolite-exposed rats at 3 months postexposure were then processed for lung tissue histopathological analyses. Sagittal lung sections were prepared for histology and evaluated by light microscopy. Histopathology results revealed that quartz particles produced foamy alveolar macrophage accumulation and patchy evidence of alveolar tissue thickening, indicating incipient phases of lung fibrosis. Exposures to the ultrafine TiO2 particles resulted in accumulation of phagocytic alveolar macrophages but no tissue thickening. Exposures to sepiolite particles produced evidence of multinucleated giant cells in airspaces and patchy evidence of minimal septal tissue thickening, suggesting the development of lung fibrosis. The severity and frequency of tissue thickening with sepiolite particle exposures appeared to be dose related. Follow-up, dedicated lung tissue histopathology studies currently are in progress to determine the reproducibility of this effect and the role of giant cells (if any) in producing minimal lung fibrosis following pulmonary sepiolite exposures.

PS 248 INFLUENCE OF PHYSICOCHEMICAL PROPERTIES OF NANO-SILICA PARTICLES ON *IN VIVO* TOXICITY.

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Nanometer-size amorphous silica particles (nano-silica), which are used as functional additives in cosmetics and foods, have quickly become essential nanomaterials in our lives. With few exceptions however, there are insufficient data available on risk assessment of nano-silica. We therefore analyzed the influence of the physicochemical properties of nano-silica on acute toxicity. We have previously shown that injection of smaller nano-silica particles into mice induced acute toxicity as particle size decreased. In the present study, we used fluorescent-labeled nano-silicas whose surface was either unmodified or modified with -NH2 and -COOH (70-nm diameter, designated SP-plain, SP-NH2, and SP-COOH, respectively), to evaluate the influence of nano-silica surface properties on in vivo toxicity and distribution. Methods) Acute toxicity tests were performed according to the following procedures. BALB/c mice were injected with 2 mg of SP-plain, SP-NH2, or SP-COOH. Survival of SP-injected mice was monitored, and histopathology tests were performed twenty-four hours after SP-injection. For image analysis, hairless mice (Hos: HR-1) were injected intravenously with nano-silicas. At indicated time points, optical imaging was performed using a Xenogen IVIS 200 imaging system. Results and Discussions) A single intravenous injection of SP-plain induced acute lethal toxicity in mice. In contrast, injection of SP-NH2 and SP-COOH into mice caused little toxicity. In vivo optical imaging of the mice revealed that SP-plain had spread throughout the liver, whereas SP-NH2 and SP-COOH accumulated around the gallbladder. These results suggest that surface properties of nano-silicas were a critical factor for its liver accumulation and expression of toxicity.

PS 249 SYSTEMICALLY-INTRODUCED NANOSCALE CERIA BIODISTRIBUTION AND TOXICITY.

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Objective: Characterize biodistribution and toxicity of nanoscale ceria that had entered blood. Rationale: Ceria was chosen as a model insoluble, stable, metal oxide tracer with extensive engineered nanomaterial (ENM) applications. Materials: A 5% crystalline aqueous ceria dispersion, mean size ~ 30 nm (PSD); primary size ~ 3 to 5 nm (HR-TEM); surface area ~13 m2/g. Procedures: The effect of saline and 10% sucrose on ceria agglomeration was assessed. Ceria was given to un-anesthetized rats i.v. (0, 50, 250 or 750 mg/kg) terminated 1 or 20 hr later to assess its biodistribution, by ICP-AES/ICP-MS cerium analysis, and potential to produce neurotoxicity, assessed by 4-hydroxy-2-nonenal (HNE), 3-nitrotyrosine (3-NT), and protein carbonyls in frontal cortex (FC), hippocampus (HC) and cerebellum (CB). Five min prior to termination anesthetized rats were given i.v. Evans blue (EB)-albumin and Na fluorescein (Na2F) as blood-brain barrier integrity markers. Results: Saline and 10% sucrose caused ceria agglomeration in vitro. Fresh blood incubated with ceria for 1 hr showed primary and agglomerated ceria by HR-TEM/STEM. Ceria $t_{1/2}$ in the rat was << 1 hr. Brain EB and Na2F increased. Tissue [Ce] was ceria dose-dependent (spleen > liver > brain > blood serum) and accounted for ~ 50% of the dose. 4-HNE increased in the HC; 3-NT changed little in FC, HC or CB; and protein carbonyls decreased in the CB. Conclusions: Ceria was cleared by peripheral tissues. Much less entered the brain. The results provide a foundation to study the impact of physico-chemical properties of ENMs on peripheral organ distribution, brain entry and resultant toxicity. Supported by the Office of the VP for Research, U KY and US EPA STAR Grant RD-833772.

PS 250 FATE OF ULTRAFINE (UFTIO2) OR FINE TITANIUM DIOXIDE (FTIO2) FOLLOWING INTRATRACHEAL INSTILLATION IN RATS.

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Nanotechnology is expanding due to the unique properties of nanoparticles. Studies report that pulmonary exposure to nano-sized particles may produce greater pulmonary effects than their larger fine–sized particle counterparts. However, differences in fate and clearance of inhaled nano-sized and fine-sized particles are unclear. This study explores differences in lung burden as well as translocation to the interstitium and the bronchial-associated lymphatic tissue (BALT) between rats exposed to UFTiO2 vs. FTiO2. Rats were exposed to an equal surface area dose of UFTiO2 or FTiO2 (0.52 mg/rat or 10.7 mg/rat, respectively). Metal analysis indicates that the amount of UFTiO2 remaining in the lung decreased by 51% from 7 to 42 days post-exposure, while the lung burden of FTiO2 decreased by only 12% . The amount of TiO2 present in the tracheo-bronchial (TBL) and thymic lymph nodes (TLN) was also assessed. Lymph node burden for UFTiO2 from 7 to 42 days post-exposure increased by 246%, while a 134% increase was observed for FTiO2. Although relative migration of UFTiO2 to the lymph nodes exceeded that for FTiO2, this difference could not account fully for the much lower lung clearance of

FTiO2 relative to UFTiO2, suggesting particle overload in rats exposed to FTiO2. Lung burden of TiO2 was measured in both lavaged and unlavaged lungs at 7 and 42 days post-exposure. The amount of TiO2 present in the lavagable fraction represents particles phagocytosed by lavagable alveolar macrophages or present as free particles in the airspaces. The non-lavagable UFTiO2 or FTiO2 fraction in the lung increased from 7 to 42 days post-exposure. With UFTiO2, the majority of the particles were non-lavagable, suggesting migration to the interstitium. In contrast, the majority of FTiO2 was found in lavagable alveolar macrophages. In summary, the results indicate that UFTiO2 migrates more rapidly to the interstitium than FTiO2 which is phagocytosed more avidly by alveolar macrophages.

PS 251 TOXICITIES OF NANOPARTICLES IN AN ENVIRONMENTALLY RELEVANT FISH MODEL.

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Toxicities of aqueous suspensions of nanoparticles were tested in early life stages of an environmentally relevant fish species, *Microgadus tomcod* (Atlantic tomcod). Embryos were exposed to graded concentrations of nanoparticles which included: fullerenes, functionalized single-walled carbon nanotubes (SWNT) and metal oxides of silver, copper, iron, nickel and Zn (Ag, Cu, Fe, Ni and Zn). Also tested were manufactured nanoparticles consisting of a fullerene shell with erbium or yttrium metal catalysts derived from various stages in their production process including soot, sludge and the finished material. Particles were dispersed in 5 ppt artificial sea water or DMSO and sonicated for one hour. Exposure began at 14 days post fertilization and continued until death or hatching. Survivorship and hatching success were evaluated. Fullerene exposure resulted in 100% mortality at 500 μg/l while SWNTs did not induce significant changes in mortality or hatching. Mortality increased with graded doses of Cu, Fe and Zn while no changes in mortality or hatching were detected with Ag or Ni at concentrations up to 10 μg/ml. The finished manufactured nanoparticles caused dose responsive toxicities that were 100% lethal at 50 μg/ml. Soot and sludge particles with both erbium and yttrium resulted in dose dependent increases in mortality but erbium-containing particles were more toxic and caused 100% mortality at 10 and 50 μg/ml for soot and sludge, respectively. These results indicate that nanoparticles containing some metals (Cu, Fe, Zn) are toxic to fish embryos. Particles containing erbium and yttrium were more harmful than those containing only carbon. Fullerenes induced mortality in these embryos but SWNTs did not. Future studies will determine if these effects are modified by exposures in natural water samples of different salinities, are conserved in a second fish species, *Fundulus heteroclitus*, as well as attempt to elucidate mechanisms of toxicity.

PS 252 GOLD AND SILVER NANOMATERIALS REDUCE THE INFLAMMATORY RESPONSE TO INJURY.

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The role of inflammation in rheumatoid arthritis (RA) is well known. Inflammation has also been implicated in the pathogenesis of other chronic diseases including cancer. The widespread role of inflammation in disease underscores an urgency to identify new and improved anti-inflammatory treatments. Chrysotherapy, injection of gold (Au) salts, has been used for decades as a standard RA treatment despite an unclear mode of action. We tested the hypothesis that nanomaterials formed upon injection of Au ions are the primary agents responsible for reducing inflammation in patients undergoing chrysotherapy. The anti-inflammatory potential of Au and silver (Ag) nanomaterials produced in organic media was measured using a larval zebrafish inflammation model. An inflammatory response can be induced in zebrafish by amputating the caudal fin. We tracked inflammatory cells by exposing transgenic zebrafish with fluorescent neutrophils to nanomaterials in solution with neutral red, a macrophage stain. We pre-exposed 3 days post fertilization (dpf) larvae to Au or Ag nanomaterials for 18 hours, then amputated their caudal fins at 4 dpf followed by immediate immersion in NOAEC nanomaterial solutions. The total area of neutrophils at the site of injury 8 hours post amputation was measured. Nine of 14 nanomaterials reduced total neutrophil area at the amputation site. Of these, 4 Ag and 1 Au nanomaterial caused a greater than 40% reduction in neutrophils. We are currently quantifying macrophage responses and investigating potential mechanisms by which neutrophils are reduced. Elucidation of the in vivo mode of action of Au and Ag nanomaterials is anticipated to contribute to the development of novel anti-inflammatory therapies. Supported by NIEHS P3000210, EPA RD-833320, T32 ES07060, Air Force Research Laboratory under agreement #FA8650-05-1-5041, and the W.M. Keck Foundation.

PS 253 EVALUATION OF SIZE-DEPENDENT BIOLOGICAL BEHAVIOR OF NANO-SILICAS.

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(Objective) Nanomaterials have recently been successfully employed in various industrial applications in medicine and cosmetics. Because nanometer-size amorphous silica particles have already been used as functional additives in cosmetics and foods, there is an urgent need for their risk assessment. We have here performed comparative toxicity studies of various sizes of silica particles (SP; SP70: 70 nm, SP100: 100 nm, SP300: 300 nm, SP1000: 1000 nm). (Method) We performed acute toxicity studies using intravenous injections of single doses (100 mg/kg) of SPs of various sizes, to evaluate their harmful effects in BALB/C mice. Six h after SP injection, serum was collected and subjected to analysis for markers of toxicity. Cytotoxicity of SP-treated HaCaT cells uated by [3H]-thymidine incorporation over 24 h. Furthermore, intracellular localization of SPs was analyzed by TEM. To analyze genotoxicity, we performed the alkaline comet assay and the Ames test. (Results and Disuccustion) Acute toxicity testing showed evidence of marked liver toxicity only in SP70- and SP100-injected mice; furthermore, SP70-injected mice died within 12 h after injection. In vitro, SP exhibited dose- and size-dependent cytotoxicity against HaCaT. TEM analysis revealed the entrance of SP70 into the nucleus. Therefore, evidence for genotoxicity of SP was investigated. When HaCaT were treated with 30 μg/ml SP for 3 h, DNA fragmentation was observed in all SP-treated groups. In the Ames test, SP treatment resulted in more than double the spontaneous mutation rate. In particular, SP70 exerted the strongest mutagenic effect. These results indicate that acute toxicity, cytotoxicity and genotoxicity tend to increase as particle size decreases.

PS 254 CLEARANCE OF THE VASCULAR INFUSED CERIUM OXIDE BY RETICULOENDOTHELIAL CELLS IN RAT.

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Objective: Biodistribution and biotransformation of the internalized nanoparticles (NP) is regulated by the strategically placed reticuloendothelial cells. The objective of this study is to determine the threshold level and time course of phagocytic cells responses in liver, lung, spleen and kidney after iv infusion of cerium oxide NP. Methods: Commercial cerium oxide from Sigma (primary particle size ≈30 nm) suspended in water was infused (50, 250, 750 mg/kg) in adult male Fischer 344 rat. They were terminated at 1 or 20 hr later. Tissue ceria level was determined by ICP-AES/ICP-MS and ROS related markers were assayed. Organs rich in RES as well as hippocampus and cerebellum were collected for histological and ultrastructural analysis. The latter included the use of HRTEM, STEM, and EDX for elemental detection of ceria. Results: A dose and time dependent retention of ceria was observed in peripheral organs, but, not in the brain. HRTEM reveal occasional ceria NP in capillaries and the associated astrocytes in the brain. By contrast, agglomerated ceria NP was abundant in the phagocytic cells of the RES. Cytotoxicity was occasionally observed in cells engulfed large NP aggregates, however, general cytoarchitecture was maintained. Conclusion: Vascular infusion of cerium oxide NP appeared to be relatively non-toxic in rat. RES cells provide the initial retention of ceria NP in a dose and time dependent fashion. However, the long-term consequences of the detained NP remain to be determined. Supported in part by the Office of the Vice President for Research, University of Kentucky and US EPA STAR Grant RD-833772.

PS 255 THE EFFECTS OF SUB-CHRONIC EXPOSURE TO INHALED NICKEL NANOPARTICLES ON THE CARDIOVASCULAR SYSTEM.

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As applications of nanotechnology continue to expand rapidly, there is an urgent need to evaluate the potential health effects of engineered nanoparticles (NPs) in occupational and environmental settings. Since there is a well-established association between inhaled ambient ultrafine particles and increased risk of cardiopul-

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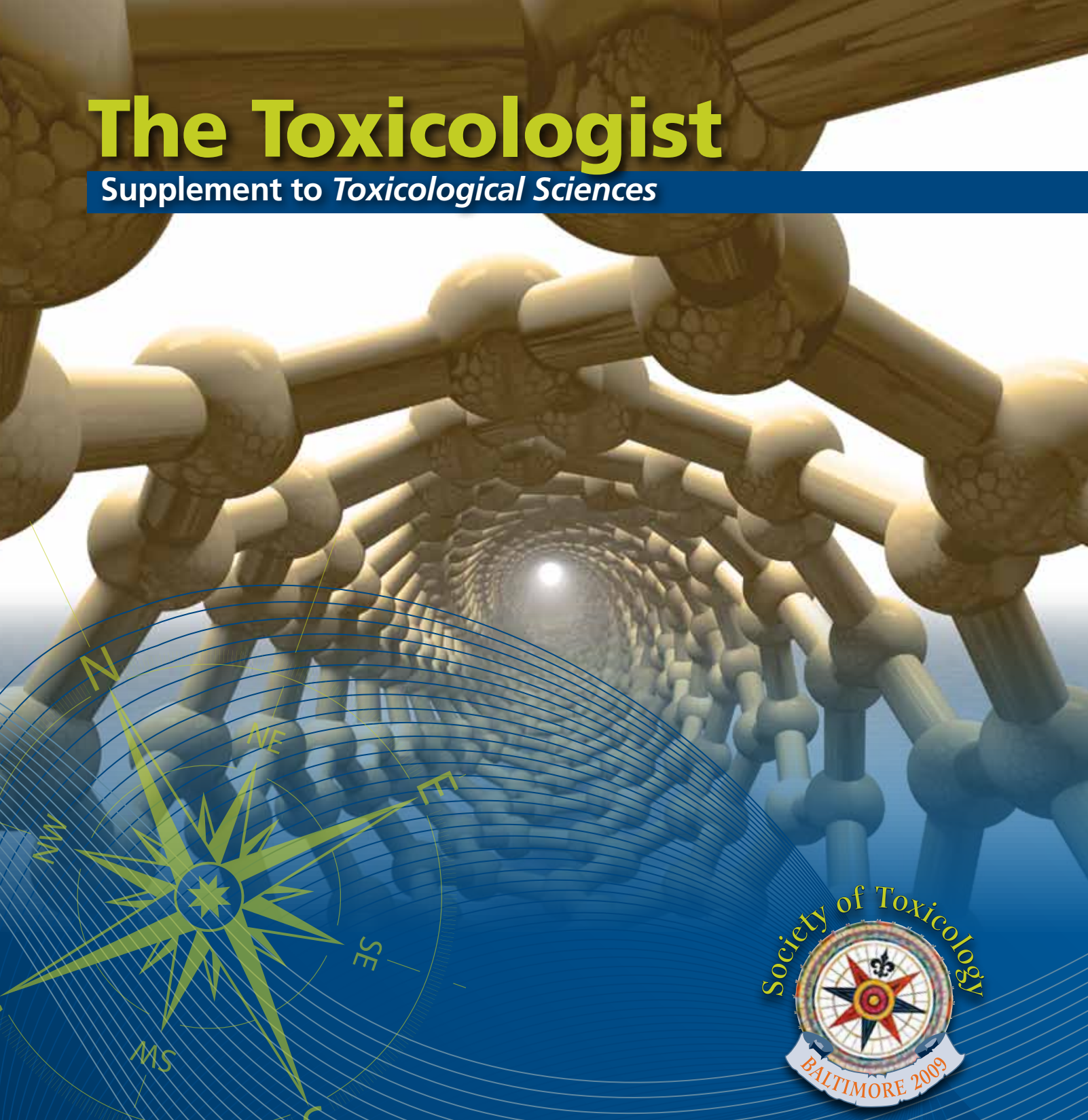

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