monary disease, it has been suggested that inhaled NPs may also induce similar effects on the cardiovascular system. This study was designed to determine if long-term exposure to inhaled nickel (Ni) NPs induced adverse effects on the cardiovascular system, resulting in exacerbation of atherosclerosis in a sensitive mouse model. Male ApoE knockout mice were exposed either to filtered air or Ni NPs at 100 µg/m3, 10% of the current occupational guideline, for 5h/d, 5d/wk for either 1 week or 5 months. Various indicators of oxidative stress and inflammation were measured in the lung and cardiovascular tissue, and plaque formation on the aorta was determined after 5m of exposure. The analyses of bronchoalveolar lavage fluid (BALF) revealed significant oxidative stress and pulmonary inflammation at both time points. This data was consistent with up-regulations of heme-oxygenase-1 (Ho-1), interleukin-6 (Il-6) and monocyte chemotactic protein-1 (Mcp-1) genes in the lung tissues. Those three genes were also up-regulated in the heart tissue after 5-m of exposure, suggesting systemic effects induced by inhaled Ni NPs. Furthermore, Mcp-1 was over-expressed in the aorta tissue, along with Cd68 and vascular cell adhesion molecule-1 (Vcam-1), after the 5-m exposure. This phenomenon coincides with increased plaque formation in the aortic arch, providing a molecular basis for the exacerbated atherosclerosis. These results suggest that inhaled Ni NPs, at occupationally relevant concentrations, can induce significant chronic effects on the cardiovascular system, including exacerbation of atherosclerosis. Further studies are needed to investigate potential mechanisms in which inhaled NPs could affect the cardiovascular system.

PS 256 SILVER NANOPARTICLE AND FULLERENE STUDIES WITH ADULT AND EMBRYONIC OYSTERS, CRASSOSTREA VIRGINICA.

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The fate and effects of nanoparticles on aquatic organisms are important environmental concerns that must be addressed as the production and uses of nanoparticles continue to increase. The purpose of these ongoing studies is to characterize the toxicity of various nanoparticle preparations (silver nanoparticles and fullerenes) on oysters, Crassostrea virginica, a common estuarine species. As filter-feeders, oysters a very valuable model species for characterizing nanoparticle bioavailability and interactions with basic cellular processes. Laboratory exposure studies were conducted with adult and embryonic oysters as well as with isolated hepatopancreatic cells. The potential for hepatotoxicity was evaluated using a lysosomal destabilization assay, and lipid peroxidation assays were used to assess oxidative damage. For the embryo assays, newly-fertilized oyster embryos were exposed to the nanoparticles and then the percent normal development after 48 hours was assessed. We used these studies to address important issues, such as relative sensitivity of embryos compared to adults, tissue distribution, and cellular accumulation and effects. Generally, embryos tend to be slightly more sensitive than adults, and isolated hepatopancreas cells were similar in toxicity to the whole oyster studies. Fluorescent confocal microscopy and atomic absorption spectrometry were used to verify the accumulation of the nanoparticles inside hepatopancreas cells and embryos. Significant accumulation of fullerenes inside lysosomes was observed supporting the model that endosomal pathways are a likely mechanism of accumulation. For the Ag nanoparticles, significant relationships were observed between tissue Ag levels and toxicity. These kinds of basic studies are essential for addressing the potential impacts of nanoengineered particles on fundamental cellular processes as well as aquatic organisms.

PS 257 INTEGRATION OF GENOMIC AND PROTEOMIC BIOSIGNATURES IMPROVES THE DISCRIMINATION OF RESPONSE TO NANOPARTICLES IN A MOUSE MODEL.

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Health risks associated with contact and consumption of nanomaterials is largely unknown and a topic of considerable interest to both manufacturers and consumers. We compared the pulmonary response to repeated exposure to single-walled carbon nanotubes, crocidolite asbestos, and ultrafine carbon black in a mouse model. Biological signatures of exposure were derived using both genome and proteome profiles though high-content analytical platforms, including microarray, global mass spectrometry-based proteomics, and multiplexed protein ELISA microarray. The goal of this study was to derive biomarkers of response from these data streams and to evaluate the screening potential of integrated biosignatures for hazard assessment. We developed a biosignature integration approach that fuses the multiple data sources at a molecular level. Probability models were derived for individual datasets by using partial least squares discriminant analysis and transformed into likelihood values associated with the probability that a particular sample was exposed to one of the defined particles or the control group. The screening potential of each biosignature was then assessed using the probability model to give insight into particle classes that biomarkers may be derived from each data source. Finally, Bayesian statistics were applied to the likelihood probability models to fuse all data streams into a single model. We find that the probability models associated with individual data types can only successfully separate less than 90% of the samples with cross-validation. However, the integrated probability model can nearly perfectly classify all samples. Using this approach, we identified a panel of biosignatures for each particle class with statistical power to predict response to particulates.
Supported by Environmental Biomarkers Initiative and ES016015

PS 258 POLYETHYLENE GLYCOL (PEG) NANOGEL AGGREGATE DRUG DELIVERY SYSTEM FOR TARGETED SYSTEMIC DELIVERY.

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The aim of this work is to develop a novel aggregated nanogel particulate (ANP) drug delivery system that targets the lung, liver or spleen using stable aggregates of a PEG nanogel. PEG nanogel particles were successfully formed by irreversibly cross-linking 8 Arm-PEG thiol polymer and a cross-linker (HBVS) in PB (pH 7.4). The effect of stirring time, cross-linker concentration, surfactant and sonication on particle size was systematically investigated. Additional experiments to grow the nanogels to micron-sized ANP were performed. Stability studies of ANPs were done at 37oC in rat plasma, PBS (pH 7.4) and PB (pH 7.4). Dynamic light scattering (DLS) and transmission electron microscope (TEM) were used for analyzing the size of ANPs. Among the parameters, sonication and the presence of surfactant (Tween 80, 0.1%v/v) were found to be critical for forming hydrogel nanoparticles. DLS and TEM results showed that after stirring the reaction mixture for a long time, the nanogel particles form aggregates due to hydrophobic interactions. Both small (20 nm) and large (≥1 µm) particles were observed in the reaction mixture. Stability studies indicated ANPs were stable in rat plasma, PBS and PB. Male Sprague-Dawley rats were intravenously administered with either HiLyte Fluor™ 750 dye alone (DYE) or ANPs covalently labeled with HiLyte750 (DYE-ANPs). Biodistribution of ANPs was determined using a Xenogen IVIS 100 Imaging System. DYE was quickly eliminated from the body, whereas DYE-ANPs accumulated in the lung within 30 minutes and the majority of the DYE-ANPs remained in the lung for 24 hours. In conclusion, these results show that it is feasible to produce nanosized PEG hydrogel particles and that these nanoparticulates can form stable micron-sized aggregates. The micron-sized ANPs are retained in the body for more than 24 hours and are predominately found in the lung.
Support: NIH/NIAMSD CounterACT Program (Award: U54AR055073)

PS 259 TISSUE-SPECIFIC ANTIOXIDANT RESPONSES OF OYSTERS, CRASSOSTREA VIRGINICA, TO SILVER NANOPARTICLE EXPOSURES.

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As nanoparticle technologies continue to grow, there is an increased potential of introduction of nanoparticles to aquatic environments. It is therefore important to characterize the potential risks to ecological receptors. Oxidative damage and impacts on antioxidant responses may impact the physiology and fitness of organisms directly, and may also increase their susceptibility to other stressors. Silver (Ag) nanoparticles are used extensively in clothing and other applications as an anti-microbial compound. The purpose of this ongoing work is to characterize the antioxidant-related responses of oysters (Crassostrea virginica) to Ag nanoparticle exposures. For these studies, gill and hepatopancreas tissues were removed from oysters following exposures to a range of Ag nanoparticle concentrations to evaluate tissue-specific antioxidant responses (e.g. glutathione and catalase concentrations). Gill and hepatopancreas tissues were also assessed for expression of metallothionein genes (MT I and II) using QT-PCR. Metallothioneins may play a dual role in ameliorating the adverse effects of metal nanoparticles by scavenging excess metal ions, and by functioning as an antioxidant by removing oxyradicals. Lipid peroxidation assays were also conducted to assess for evidence of tissue damage associated with oxyradial production. While the results indicated little direct tissue damage following short term exposures to Ag nanoparticles, tissue-specific alterations of antioxidant responses were observed.

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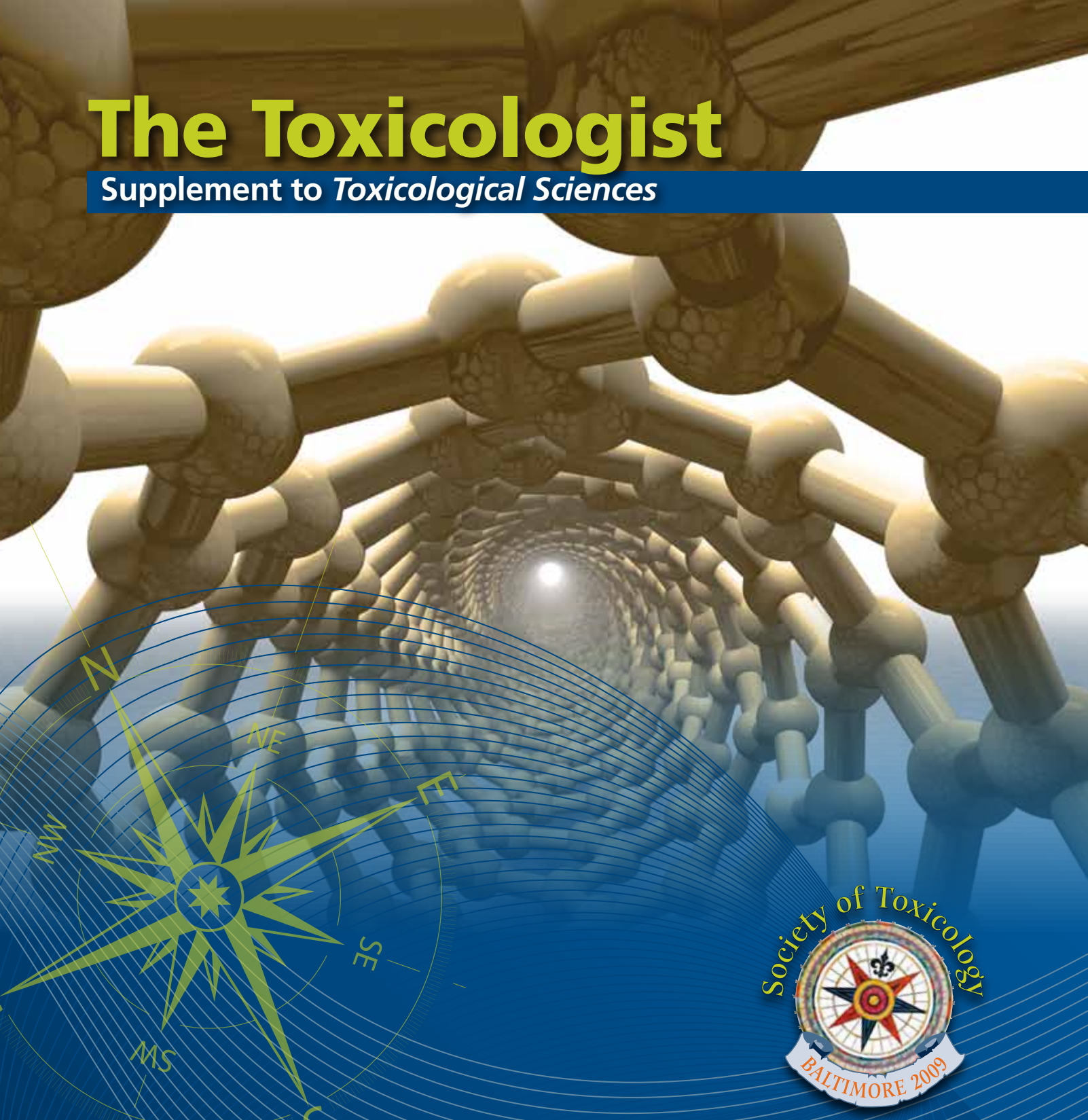

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