posure, blood chemistry was analyzed, and lungs were subjected to pathologic analysis. These mid-study data show elevated pCO2 and lowered blood pH and pO2, which correlate with upper airway necrosis and measurements of pulmonary function in the 24-48 h after 3 mg/kg HD exposure. The period between 3 and 7 wks presented a significant challenge because approximately 15% of our high exposure group (3 mg/kg) and 10% of our 6-month LCT50 group (2.25 mg/kg) either died suddenly or required withdrawal from the study during this timeframe. Alveolar exudates, edema, and inflammation peaked at 3 wks and correlated with compensatory changes in pulmonary function and/or respiratory distress. Animals that demonstrated respiratory distress at 3 wks were more likely to die later in the study. Importantly, this indicates that 3-7 wks post-exposure may be a crucial window in the progression of HD inhalation injury, and that therapeutic intervention prior to and concurrent with this time-point may offer the best approach. This study provides the first long-term examination of HD-induced lung injury and systemic effects.

PL 1756 THERAPEUTIC EFFICACY OF CATALYTIC ANTIOXIDANT AEOL 10150 IN ATTENUATING SULFUR MUSTARD ANALOG 2-CHLOROETHYL ETHYL SULFIDE-INDUCED SKIN INJURY.

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Sulfur mustard (SM), a bifunctional alkylating chemical warfare agent, inflicts devastating injury with delayed vesication of the skin. With the goal to elucidate mechanism/s of SM-caused skin injury as well as to screen for therapeutics, our recent studies with SM analog 2-chloroethyl ethyl sulfide (CEES) have established CEES-induced injury biomarkers in skin epidermal cells and SKH-1 hairless mouse. These studies in SKH-1 hairless mouse skin with CEES and other published reports with SM and CEES have indicated a role for oxidative stress in skin injury caused by these agents. Accordingly, employing our CEES-induced skin injury model, here we determined if treatment with the metalloporphyrin catalytic antioxidant AEOL 10150 could attenuate CEES-induced skin injury. AEOL 10150 treatment of mouse epidermal JB6 cells 1 h post 0.25 mM CEES resulted in over 50% ($p<0.05$) reversal of CEES-induced decrease in cell viability, DNA synthesis inhibition and DNA damage. Similar treatment in human epidermal HaCaT cells also showed significant ($p<0.05$) reversal in CEES-caused decrease in DNA synthesis. Animal efficacy studies were carried out with AEOL 10150, wherein SKH-1 hairless mice were treated with topical formulation (TF) and/or subcutaneously (SC) with 5 mg/kg AEOL 1 and every 4 h after 4 mg/mouse CEES exposure for up to 12 h. Treatment with both TF and SC resulted in over 50% ($p<0.05$) reversal in CEES-caused increase in both skin bi-fold thickness and myeloperoxidase (MPO) activity. Studies are currently underway to further determine the rescue effects of AEOL 10150 employing other established CEES-induced inflammatory and oxidative stress biomarkers from our studies. Nevertheless, results from the current study demonstrate promising therapeutic efficacy of AEOL 10150 in rescue from SM-caused skin injury, warranting further investigation and optimization of its treatment potential.

PL 1757 NANOSILVER MOVEMENT THROUGH BIOLOGICAL BARRIERS RELATES TO PHYSICOCHEMICAL PROPERTIES.

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Linking the physicochemical (PC) properties of engineered nanomaterials (NM) to their biological activity is critical for identifying their (toxic) mode of action, and developing appropriate and effective risk assessment guidelines. Particle surface charge (zeta potential), surface coating and "redox" activity are PC properties thought to influence NM uptake and movement (i.e., translocation) through cellular barriers. Nanosilver (nanoAg) is used in water purification, antiseptics, biocides, medical devices and a variety of consumer and manufactured products. Efforts to reduce its environmental burden and enhance its bioavailability favor "green chemistry" synthesis and the surface coating of particles with benign materials. Since ingestion is a dominant route of exposure, studies were conducted to examine how such PC properties affected nanoAg movement across in vitro models of biological barrier cells. Monolayers of human intestinal (Caco-2) or rat blood brain barrier (RBE4) cells were exposed (3.12-6.25 ppm) to commercially available, capped (citrate or PVP) or uncapped nanoAg particles (10nm and 70 nm). Another sample of nanoAg was synthesized, using a borohydride reduction method, and tested as uncoated particles (50-75 nm) or those coated with glutathione (GSH) or green tea (GT). Microscopy (confocal, TEM) indicated that nanoAg particles translocated barrier cells rapidly (<15 min) and without disrupting their tight junctions. Changes in transcellular electrical resistance (TERS) suggested that surface coatings (citrate>PVP; GSH=GT), an electronegative zeta potential and low aggregate size were PC properties that enhanced nanoAg's cellular movement. Future studies will use high through put techniques to confirm these relationships and will also examine how different surface coatings of nanoAg particles affect their cellular uptake, retention and clearance. (This abstract has been reviewed by NHEERL and does not necessarily reflects EPA policy)

PL 1758 TOXICITY, CELLULAR UPTAKE, AND DIFFERENTIATION OF HUMAN ADULT STEM CELLS EXPOSED TO SILVER NANOPARTICLES.

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Orthopedic implantation of tissue engineered bone grafts are becoming a viable alternative to autografts and allografts. Grafts are grown by seeding adult, patient-specific adipose-derived mesenchymal stem cells (hASCs) onto a degradable scaffold and chemically inducing the cells down their osteogenic lineage. A limitation of orthopedic implants pertains to surgical site infections, which result in graft failure and secondary surgery. Silver nanoparticles (Ag-nps) have antibacterial properties that could be incorporated into tissue engineered bone grafts as a preventative measure against surgical site infections. The purpose of this study was to assess the toxicity, cellular uptake, and effect on differentiation of Ag-nps in hASCs. The hASCs were isolated from adipose tissue from elective plastic surgery procedures and either cultured to maintain their self-renewal in growth medium or chemically induced down their adipogenic or osteogenic pathways. During culture, hASCs were exposed to either a single or 7-day repetitive dose of 0.1, 1.0, and 10.0μg/ml of 20nm spherical Ag-nps. Following exposure, toxicity was assessed using alamarBlue viability assay, which showed a significant decrease ($p<0.05$) in viability for both single and repetitive exposures for both osteogenic and adipogenic cells at 1.0μg/ml but not for self-renewing cells. Differentiation of hASCs was examined by Oil red-O (lipid) and alizarin red-S (calcium) and showed no change in differentiation following exposures at any concentration for each of the osteogenic, adipogenic, or self-renewal cells. Transmission electron microscopy was utilized to detect ultrastructural changes and/or differences in uptake of Ag-nps by any of the cells; all cell types were shown to take up the Ag-nps without causing ultrastructural alterations after exposure to all concentrations of Ag-nps. (Supported by US-AFOSR FA 9950-08-1-0182)

PL 1759 PULMONARY TOXICITY FOLLOWING INTRATRACHEAL INSTILLATION OF DISPERSED SILVER NANOPARTICLES IN RATS.

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Silver nanoparticles (Ag NPs) are emerging as one of the fastest growing categories of manufactured materials in the nanotechnology industry, which may lead to an increased risk of worker exposure to this nanomaterial. The goal of these studies was to characterize pulmonary toxicity of Ag NPs using an in vivo model. Ag NPs were 20 nm in diameter with a polyvinylpovidone coating (PVP, 0.3% wt) (NanoAmor, Inc.). Specific surface area was measured to be 7.54 ± 0.103 m^2/g. Ag NPs were suspended in a dispersion medium (DM, phosphate-buffered saline + 0.6 mg/ml rat serum albumin + 0.01 mg/ml dipalmitoyl phosphocholine) and sonicated. Aggregate size was evaluated in DM using dynamic light scattering and average agglomerates were ~150 nm. On day 0, Sprague-Dawley rats were intratracheally-instilled with Ag NPs in DM at doses of 9.35, 37.6, 112, 224, or 449 μg, SiO_2 at a dose of 449 μg (positive control), or DM alone (vehicle control). Rats were humanely sacrificed 1, 7, 14, and 28 days post-exposure, the right lung was lavaged, and lung-associated lymph nodes (LALN) were removed for analysis. Lavage fluid parameters (lactate dehydrogenase and albumin), cellular influx into the lung (macrophage, neutrophil, and lymphocytes), and LALN cell number and phenotype were evaluated to assess lung injury, inflammation, and immune response. Indices of lung injury and inflammation were significantly elevated in a dose-dependent manner at days 1 and 7, which exceeded the positive control at the highest dose. By day 14, only the rats exposed to 449 μg Ag NP dose had significantly elevated parameters similar to that of the positive control which persisted out to day

28. There was a dose-dependent increase in LALN cell number beginning at day 7 and continuing in the 3 highest doses out to day 28. There was also a dose-dependent increase in lymphocytes in the lavage fluid. Collectively, these data demonstrate the Ag NPs have the capacity to induce pulmonary injury and inflammation, as well as alter immune responses in the lung.

PL 1760 OPTIMIZATION OF HIGH-THROUGHPUT NANOMATERIAL DEVELOPMENTAL TOXICITY TESTING IN ZEBRAFISH EMBRYOS.

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Nanomaterial (NM) developmental toxicities are largely unknown. With an extensive variety of NMs available, high-throughput screening methods may be of value for initial characterization of potential hazard. We optimized a zebrafish embryo test as an in vivo high-throughput assay for NM developmental toxicity by assessing 5 zebrafish embryo rearing solutions for their effects on NM aggregation as well as embryo development. We compared the aggregation of NM in 5 solutions [10% Hank's, Danieau, 60 mg/L artificial seawater (ASW), full strength (1X) and 10% EPA moderately hard reconstituted water (MHRW)] by dynamic light scattering analysis. Silver nanoparticles (nano-Ag) coated with citrate-, polyvinylpyrrolidone (PVP)-, or gum arabic (GA) were suspended in these 5 solutions as well as deionized water (ddH_2O) for 24 h. All nano-Ag aggregate sizes in both MHRWs were similar to that in ddH_2O. GA-coated nano-Ag aggregates were largest in Hank's, followed by Danieau, and distantly by ASW. PVP-coated 10 nm nano-Ag and citrate-coated nano-Ag aggregate sizes were slightly larger in Hank's than in ddH_2O. PVP-coated 25 nm nano-Ag sizes were similar in all 5 solutions and ddH_2O. Overall, the aggregates were largest in Hank's, followed by Danieau, ASW, and smallest in MHRWs and ddH_2O. This confirms that aggregation is generally more severe in ion-rich solutions. We reared zebrafish embryos from days 0-6 post-fertilization in these 5 solutions without NM, and used death, malformations, non-hatch and days to hatching as health endpoints. All solutions had the same death and malformation scores. Compared to Hanks, all solutions, except 1X MHRW, delayed hatching. 1X MHRW had fewer non-hatch than 10% MHRW, although not significant. 1X MHRW appears to be optimal for NM testing in zebrafish embryos for being ideal for embryo development and least likely to promote NM aggregation. *This abstract does not necessarily reflect U.S. EPA policy.*

PL 1761 TOXICITY OF INTRANASALLY-ADMINISTERED SILVER NANOPARTICLES.

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Silver nanoparticles (SNP) are present in numerous consumer and industrial products, due in part to their anti-bacterial properties. Previous *in vitro* studies showed that SNP can adversely affect the blood brain barrier and up-regulate inflammatory cytokines. When injected i.p., 25 nm SNP altered expression of inflammatory and oxidative stress genes in several brain regions. Given current interest in disinfectant sprays containing SNP, we used a more physiologically-relevant route of exposure, intranasal instillation, to examine toxicity endpoints in mice. SNP (25-nm diameter, dispersed in sterile ultrapure water) were intranasally instilled in anesthetized male C57BL/6J mice (100 or 500 mg/kg b.w.). Endpoints measured 1 or 7 days after treatment included histopathology, MAC-3 immunohistochemistry (macrophage marker), reduced glutathione (GSH) levels, and gene expression changes. Body weight and general behavior/activity of treated mice were not affected at either dose or time point. Aggregated SNPs were present in the nasal cavity and associated with epithelial erosions, inflammatory cell infiltrate, and exudates at both doses and time points. One day after exposure, aggregated SNP were visible in spleen, associated with loss of cellularity in the red pulp, and co-localizing with MAC-3 immunoreactivity. Occasional particle aggregates were seen in the lung and renal medulla, with no histopathological changes in structure. Brain and liver histological sections were within normal limits at both doses and time points. GSH, an indicator of oxidative stress, was elevated in blood and nasal epithelia, but not different from control in other tissues. qRT-PCR showed elevated expression of *Kim1*, a marker of kidney damage, indicating that kidney, as well as nasal epithelia and spleen, may be the most significantly impacted tissues following intranasal exposure to 25 nm SNP. Supported by P30-ES06096 and T32-ES010957.

PL 1762 RECOVERY FROM SILVER NANOPARTICLE EXPOSURE-INDUCED LUNG FUNCTION CHANGES IN SPRAGUE-DAWLEY RATS.

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In the previous study, the lung function, as indicated by the tidal volume and minute volume, decreased during the 90 days of exposure to silver nanoparticles (AgNPs), and these decreases in the lung function were also accompanied by inflammatory lesions in the lung morphology. Accordingly, this study attempted to investigate the recovery of lung function changes in rats after a 12-week AgNP inhalation exposure to elucidate the process of lung function recovery after cessation of NP exposure. The male and female rats were exposed to silver nanoparticles (14-15 nm diameter) at concentrations of 0.67×10^6 particles/cm^3 (low dose), 1.41×10^6 particles /cm^3 (middle dose), and 3.24×10^6 particles /cm^3 (high dose) for 6 h/day in an inhalation chamber for 12-weeks. Then the rats were allowed to recover from the AgNP exposure. The lung function was measured every week after the daily exposure and after the cessation of exposure, and the animals sacrificed after the 12-week exposure period, and 1 and 3 month after the cessation of exposure. There was exposure related lung function decreases in the male rats during exposure and 3 month recovery period. However, female rats did not show a consistent lung function decrease during exposure period or after the cessation of exposure. Our results suggest that the lung function changes induced by AgNP exposure could be persistent even after recovery period.

PL 1763 CLEARANCE OF ACCUMULATED SILVER AFTER CESSATION OF SILVER NANOPARTICLE (AGNP) EXPOSURE IN TISSUES OF SPRAGUE-DAWLEY RATS.

J. Lee[1], I. Yu[1], Y. Kim[2], K. Song[3], H. Ryu[3], J. Sung[3], M. Kwon[1], J. Park[4], J. Lee[5], H. Park[6], C. Kang[1] and D. Marshak[7]. *[1]Hoseo University, Asan, Republic of Korea, [2]KT&G, Daejeon, Republic of Korea, [3]Korea Conformity Laboratories, Incheon, Republic of Korea, [4]Chung-Ang University Seoul, Republic of Korea, [5]Occupational Lung Diseases Institute, COMWEL, Ansan, Republic of Korea, [6]Korea Research Institute of Standards and Science, Daejeon, Republic of Korea and [7]PerkinElmer, Boston, MA.*

Silver nanoparticls (AgNPs) can be distributed many tissues after oral or inhalation exposure. Clearance of the distributed AgNPs from the tissues is very important to understand the fate of AgNP in vivo. Accordingly, to clarify the clearance of the tissue Ag concentration after the cessation of AgNP oral exposure, Sprague-Dawley rat were assigned to 3 groups: control, low dose (100 mg/kg BW) and high dose (500 mg/kg BW), and administered AgNPs (average diameter 25 nm) by gavage for 28 days. Then the rats were allowed to clear by ceasing the AgNP exposure. The tissue Ag contents in the most tissues such as liver, kidneys, spleen and blood decreased gradually from 1, 2 and 4 months after the cessation of exposure, indicating clearance of AgNPs. In contrast, the silver tissue concentrations in the brain and testis did not decrease rapidly as compared with other tissues even 4 month after the cessation of exposure, suggesting the accumulated AgNP may not be easily transported out from the tissues. Therefore, the tissues with biological barriers such as the blood bran barrier and blood testes barrier may play important role in clearance of AgNPs from the respective tissues.

PL 1764 PREMATURE CELLULAR SENESCENCE-INDUCED BY NANOPARTICLE EXPOSURE: A NOVEL APPROACH TO NANOTOXICITY TESTING.

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Cellular senescence is the natural aging of cells, but premature cellular senescence can be induced by exposure to toxicants. Once a cell enters senescence it will no longer proliferate and it undergoes characteristic changes in phenotype and tissue specific changes in function. In this study we tested the utility of premature cellular senescence as an indicator of nanoparticle toxicity. A549 alveolar epithelial cells were cultured for 2 hours in growth medium containing 10 or 100 nm silver or gold nanoparticles, then allowed up to 6 days of recovery in normal growth medium. Premature cellular senescence was confirmed by an increase in senescence-associated-β-galactosidase (SA-β-Gal) activity, morphological changes, cell cycle arrest, and loss of proliferative potential. The incidence of senescence was cor-

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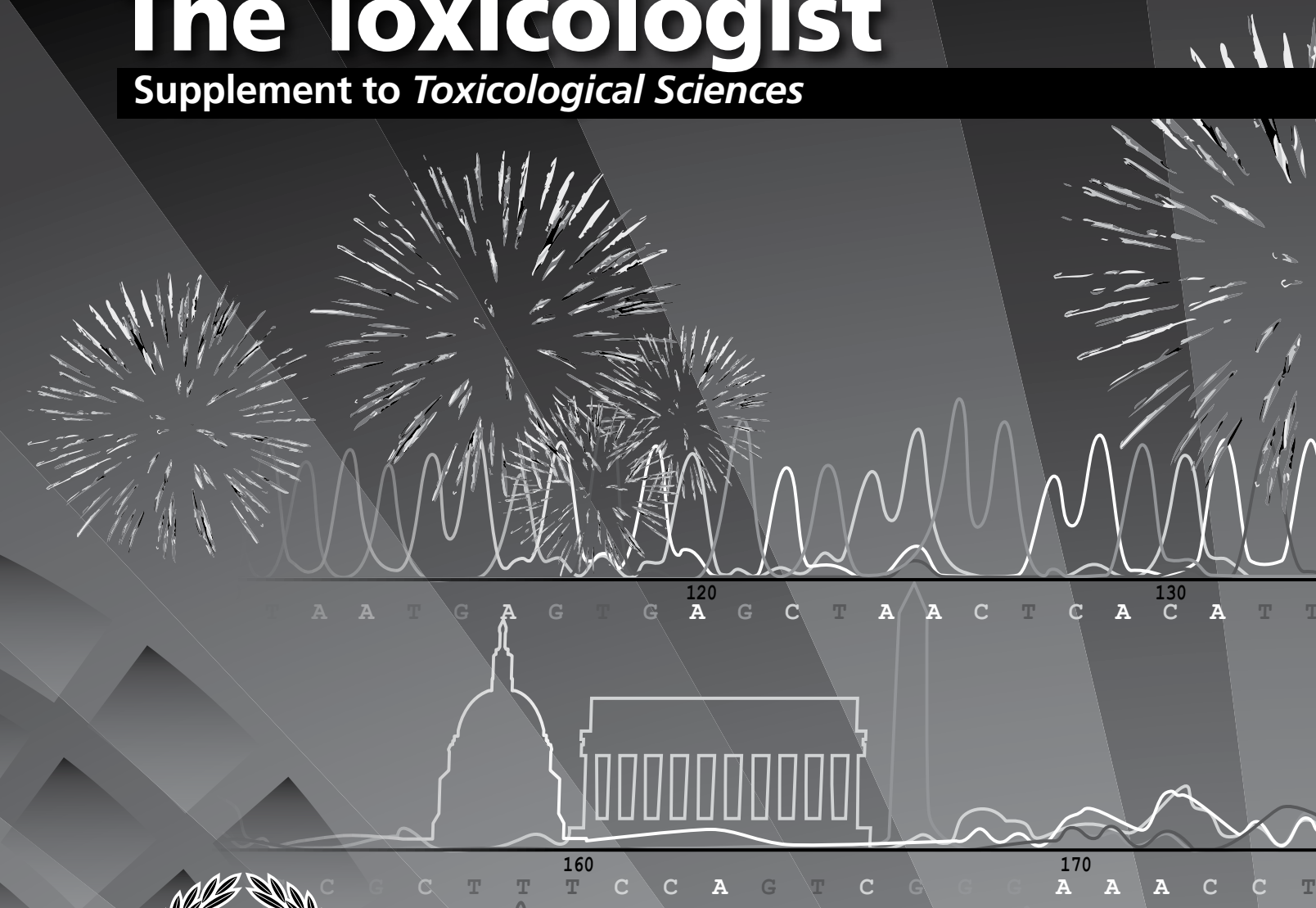

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 50th Annual Meeting of the Society of Toxicology, held at the Walter E. Washington Convention Center, March 6–10, 2011.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 578.

The issue also contains a Key Word Index (by subject or chemical) of all the presentations, beginning on page 606.

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