1482 CADMIUM EXPOSURE ASSESSMENT USING TOTAL DIET STUDY AND PROBALISTITC MONTE CARLO SIMULATION IN A CADMIUM-POLLUTED REGION, JAPAN.

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Due to past mining or volcanic activities, there exist cadmium-polluted regions in Japan. Past total diet studies in non-contaminated areas revealed that daily cadmium intake was 45 μg/day in 1977, decreased gradually to less than 30 μg/day since 1985 and sustained at about half of Provisional Tolerable Weekly Intake (PTWI) of 7 μg/kg bw per week. We conducted dietary Cd exposure assessment among 963 farmwomen at a contaminated area in Japan using both market basket method and probabilistic statistical method. Food intakes used were derived from the JMETS study conducted in this district during 2002-2005. Cd contents of food items, including agricultural, marine products, and processed foods, were collected at local food markets and measured their Cd concentrations by ICP-MS. The Monte Carlo simulation was used to estimate distributions of their cadmium intake. When no maximum levels were assigned, the mean, median, and 95 percentile of the estimated cadmium intake were 7.16, 6.29, and 14.20 (μg/kg bw/week), respectively. By market basket method of total diet study, the arithmetic mean, median, and range of cadmium intake were 7.40, 6.64, (1.25-38.98) μg/kg bw/week. Contribution from rice is 51-56% of total Cd intake in this district. The elder age groups showed high intake due to their dietary habits, such as eating more rice and marine products. The probabilistic intake estimate showed slightly lower than estimate by market basket method. However, in this Cd-polluted district, approximately half of study individuals are above PTWI by JECFA. But we could not demonstrate any statistically significant adverse health effects by Cd among the individuals, as previously reported in 44th SOT Meeting (2005) and Environ Res. 97 (1): 83-92, 2005.

1483 DETERMINATION OF A SOUTHERN CALIFORNIA REGIONAL BACKGROUND ARSENIC CONCENTRATION IN SOIL.

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Background metals in soil can prove problematic for risk assessment purposes because metals detected at a site may be comprised of naturally occurring metals, regional anthropogenic contributions or a site-specific release. Arsenic is especially problematic since the risk-based soil concentration is 100-times below typical ambient concentrations. The California Department of Toxic Substances Control (DTSC) established a regional background arsenic concentration in soil that can be used as a screening tool for sites throughout southern California. The term "background" collectively refers to both naturally occurring and anthropogenic ubiquitous concentrations in shallow soil. Data were derived from completed Preliminary Environmental Assessment (PEA) reports for proposed school sites. Site data were combined for each county in southern California, including Los Angeles, Orange, Riverside, San Bernardino and San Diego counties. Los Angeles County had the largest number of sites (19 school sites) and arsenic data points (1097 samples) and will serve as the model for the statistical derivation of background arsenic. A probability plot of the arsenic data clearly demonstrated a classical, lognormal distribution from which outliers were determined using the box plot. The summary statistics for the arsenic data set, excluding the outliers, were calculated and the upper-bound arsenic concentration estimated using both the 95% confidence limit of the 99th quantile of the arsenic data set and a distribution-free, nonparametric analysis. Both statistical methods resulted in an upper-bound arsenic concentration of approximately 12 mg/kg for Los Angeles County. Using the same approach, the upper-bound arsenic concentrations were similar for each of the other southern California counties, resulting in an upper-bound estimate of 12 mg/kg for arsenic in southern California. A similar evaluation is being conducted by DTSC for northern California sites in order to derive arsenic screening levels State-wide.

1484 RESEARCH ON TOXIC CHEMICALS IN THE GREAT LAKES: DEFINING STUDIES TO HELP ENSURE HEALTHY PEOPLE IN EVERY STAGE OF LIFE.

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ATSDR in partnership with the Great Lakes state health departments, academic institutions and federally-recognized tribal governments has been conducting epidemiological studies in the U.S .Great Lakes (GL) region to determine exposure to persistent toxic substances (PTSs) which include PCBs, dioxins, methylmercury, pesticides and others via consumption of contaminated GL fish, and to assess the potential for adverse health outcomes in vulnerable populations in the Great Lakes states.

Overall results of these indicated that for the studied populations that: 1) mothers who had serum PCB levels of 4.7 ppb were less likely to have a male child than mothers with the lowest serum level of 0.7 ppb, 2) a significant decline in birth weight of

approximately 500grams in newborns of mothers who had PCB serum levels > 25 ppb,

3) mothers who consumed two or more fish meals per month the risk of a male child having a birth defect was significantly elevated, 4) women who delivered before 35 weeks gestation were more likely to have mercury levels at or above the 90th percentile level, and 5) in women, but not in men, higher PCB serum levels were associated with increased incidence of diabetes. These research findings are of public health concern. The populations identified are at risk because of their elevated exposures and/or their intrinsic sensitivity to PTSs. ATSDR and its partners has developed prudent public health interventions and risk communication tools to interdict future exposure to toxic chemicals in these vulnerable populations. The health data will be discussed and intervention strategies will be described.

1485 AN *IN-VITRO* ASSESSMENT OF A NANOSCALE, REDOX-SENSITIVE ANTIOXIDANT DELIVERY SYSTEM'S IMPACT ON OXIDATIVE DAMAGE.

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A nanoscale antioxidant delivery system (ADS) was synthesized to target and ameliorate cellular oxidative damage resulting from the production of reactive oxygen species (ROS). High levels of ROS are associated with many disease states and conditions including airway inflammatory responses such as asthma and exposure to reactive airborne nanoparticles. This delivery system incorporates folic acid conjugated to poly(ethylene glycol), for receptor targeting through receptor-mediated uptake, and disulfide linked glutathione (GSH) for redox-triggered antioxidant release. Toxicity of the synthesized nanoparticles was assessed using a Promega (Madison, Wi) CellTiter-Glo viability assay. The uptake of the ADS by Lipopolysaccharide (LPS) stimulated RAW 264.7 cells (murine macrophage) was assessed using both flow cytometry and fluorescence microscopy. Oxidative status was assessed by measuring oxidized dichlorofluoroscein-diacetate (DCF-DA) and dihydroethidium (DHE) as indicators of H_2O_2 and $O_2^{\bullet -}$ concentration. F_2-Isoprostanes, biomarkers for lipid peroxidation, were also quantified using negative-ion GC-MS. RAW 264.7 cells treated with the GSH containing delivery system showed improved viability over both control cells (37.5%) and cells treated with a delivery system which did not contain GSH (40.7%). Both H_2O_2 and $O_2^{\bullet -}$ levels were reduced indicating decreased levels of intracellular ROS. Cells treated with the GSH containing antioxidant system also resulted in a 67.8% decrease (from 732 to 235 ng/mg protein) in F_2-Isoprostane concentrations. These results demonstrate the efficacy of targeted delivery of GSH on intracellular ROS concentrations in RAW 264.7 cells. This research also suggests that the conjugation of GSH and other antioxidants on the surface of engineered nanoparticles may reduce cellular oxidative damage and overall cytotoxicity.

1486 COMPARISON OF THE BIOLOGICAL ACTIVITY BETWEEN ULTRAFINE AND FINE TIO2 PARTICLES IN RAW 264.7 CELLS ASSOCIATED WITH OXIDATIVE STRESS.

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Ultrafine or fine TiO2 particles are widely used in the production of white pigments, sunscreens and cleanup techniques. However, currently the knowledge is deficient of cellular response to these particles. The study evaluated and compared the biological activity of ultrafine and fine TiO2 particles in RAW 264.7 macrophages according to an oxidative stress paradigm. We found that in vitro exposure of macrophages with ultrafine or fine TiO2 in the range of 0.5-100 μg/ml did not significantly alter cell viability. Ultrafine TiO2 enhanced intracellular generation of reactive oxygen species (ROS) to a greater extent than fine TiO2 at each exposure dose. Ultrafine TiO2 induced ERK1/2 activation in a dose-dependent manner,

while the fine-TiO2-induced changes were minimal. Phosphorylation of ERK1/2 occurred following 10 min exposure to higher doses of ultrafine TiO2 (above 25 μg/ml). Similarly, ultrafine TiO2 exposure significantly enhanced TNFα and MIP-2 secretion in a dose-dependent manner and its potency was higher than fine TiO2. These findings suggest that at relatively low doses of the particles, ultrafine TiO2 has greater biological activity associated with oxidative stress, such as ROS generation, ERK 1/2 activation, and proinflammatory mediator secretion, in RAW 264.7 macrophages than fine TiO2.

1487 EVALUATION OF *IN VITRO* CELL CULTURE SYSTEMS TO ACCURATELY PREDICT THE PULMONARY TOXICITY OF INHALED OR INSTILLED FINE OR NANO ZINC OXIDE PARTICLES.

D. B. Warheit, M. B. Golt, C. M. Sayes and K. L. Reed. *DuPont Haskell Laboratory, Newark, DE.*

Previous studies have reported little correlation between the relative toxicity of particle-types when comparing lung toxicity rankings following in vivo exposures compared to in vitro, cell culture exposures. This study was designed to assess the capacity of in vitro screening assays to predict in vivo pulmonary toxicity of fine (111 nm, 9.6 m2/g) or nanoscale zinc oxide (90 nm, 12 m2/g) particle-types in rats. Rats were exposed by intratracheal instillation to 1 or 5 mg/kg of nanoscale or fine size zinc oxide particle-types. For the inhalation studies, rats were exposed to aerosols of 25 or 50 mg/m3 for 1 or 3 hours. For both types of in vivo studies, lung inflammation, cytotoxicity, cell proliferative and histopathological endpoints were assessed at several time points postexposure.
For the in vitro component of the study, three different culture conditions were utilized. Cultures of 1) rat lung epithelial cells, 2) primary alveolar macrophages, as well as 3) alveolar macrophage – L2 lung epithelial cell co-cultures were incubated with fine or nano ZnO, and the culture fluids were evaluated for cytotoxicity endpoints (LDH, MTT) as well as inflammatory cytokines (MIP-2, TNF-α, and Interleukin-6) at different time periods – 1 hr, 4 hrs, 24 hrs or 48 hrs.
In vivo exposures to instilled or inhaled fine or nanoscale zinc oxide produced a "metal fume fever" type of response, with extended but transient lung inflammatory or cytotoxic responses which were resolved after a few days postexposure. Alternatively, in vitro exposures to fine or nanoscale ZnO produced cytotoxic responses at 4 or 24 hrs but not 1 or 48 hrs incubation in L2 cells and co-cultures, but not in macrophages. Cytokine generation (MIP-2, TNF-α, and Interleukin-6) in ZnO-exposed cells was not significantly different from controls at any dose or time period. To summarize, the comparisons of in vivo and in vitro toxicity measurements demonstrated little correlation, particularly when considering many of the variables assessed in this study.

1488 ASSESSING THE PULMONARY IMPACTS OF TWO SELECTED AMORPHOUS SILICA NANOPARTICLE SIZE RANGES IN RATS FOLLOWING INHALATION EXPOSURES.

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Few data have been generated on the pulmonary toxicity of inhaled nanoparticles per se. This research was designed to systematically evaluate the role of nanoparticle size on pulmonary toxicity parameters in rats exposed by inhalation to smaller (30 nm) vs. larger (70 nm) sized silica nanoparticles. The experimental design was divided into four parts: 1) nanoparticle generation, 2)nanoparticle characterization, 3) stability over time, and 4) mammalian pulmonary hazard effects. To initiate this experimentation, aerosol nanoparticle test materials of untreated, as-synthesized, amorphous SiO2 in two different nanoparticle size range populations (i.e. number distributions centered at d50 = 30 nm and d50 = 70 nm) were developed. Each of the size populations was synthesized in situ and upstream of the inhalation apparatus. The aerosolized nanoparticle populations were tested for stability over time by measuring chamber temperatures and humidity. Groups of 5 animals were exposed to 1.5 × 10E7 particles/cm3 for either 1 × 5 hour period or for 3 × 5 hour periods over 3 consecutive days to establish a dose-response relationship at each post-exposure time-point (24 hrs, 1 wk, and 1 mo). We also conducted a flow cytometric assessment of induction of micronuclei in peripheral blood samples. During the nanoparticle aerosol exposures to rats, particle characterization data was collected at t=0, 1, 2.5, 4, and 5 hours. Characterization results demonstrated precise size populations with 5% size distributions; chemical composition (SiO2), surface charge (-30 mV), and amorphous structure remained identical over the exposure duration. Subsequent post-exposure pulmonary toxicity results (including BAL fluid inflammatory and cytotoxicity endpoints) demonstrated little difference in response to the two different sized nanoparticle-types when comparing exposures to the two different nanoparticle size populations or to sham-exposed air controls. Genotoxicity and lung tissue analysis studies are on-going.

1489 MECHANISMS OF NANODIAMOND PARTICLE INDUCED IL-8 EXPRESSION IN HUMAN AIRWAY EPITHELIAL CELLS.

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Nanodiamond particles (NDP) prepared by detonation under confined conditions have a number of industrial and analytical applications. Previous *in vitro* studies have reported NDP to be biologically inert with negligible cytotoxicity, implying that they are potentially suitable for biomedical applications such as drug delivery. Separate studies have shown that elemental carbon particles that simulate the carbonaceous core of combustion derived airborne particulate matter can induce inflammatory responses in the lung through the generation of reactive oxygen species. To assess the respiratory effects of exposure to NDP, we examined its effects on IL-8 expression by human airway epithelial cells (HAEC) *in vitro*. Four hour exposures of HAEC to 66 μg/ml NDP (average particle diameter of 5 nm and surface area ~300 m^2/g) resulted in IL-8 mRNA increases up to 100-fold over resting levels and was accompanied by up to 13-fold increases in IL-8 protein levels in the media. Transfections with IL-8 promoter constructs showed that exposure to NDP increases IL-8 transcriptional activity. Separate experiments indicated that HAEC avidly take up NDP and that this uptake can be blocked with cytochalasin D. Furthermore, cytochalasin D also abrogated NDP induced IL-8 mRNA levels. NDP-induced IL-8 expression was blunted by overexpression of catalase, and to a lesser extent SOD, in HAEC. We conclude that NDP induce IL-8 expression via a transcriptional mechanism that requires particle uptake and involves the formation of reactive oxygen species. These data are evidence of an inflammatory response to NDP exposure in human lung cells. THIS ABSTRACT OF A PROPOSED PRESENTATION DOES NOT NECESSARILY REFLECT EPA POLICY.

1490 GOLD NANOPARTICLES INDUCE OXIDATIVE DAMAGE IN LUNG FIBROBLASTS *IN VITRO*.

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Scope: Nanomaterials are found to have many uses and potential applications in the fields of biology and medicine. Gold nanoparticles (AuNPs) in particular are being developed as novel gene and drug delivery agents as well as various imaging systems due to due to the inertness of bulk gold. Recent research findings have brought to light concerns over the safety of nanomaterials and long-term adverse effect of their use. In this study, we exposed AuNPs to lung fibroblast in vitro and studied the adverse effects of the nanoparticles to the cells.
Experimental procedures: MRC-5 human lung fibroblast cell line was exposed to 20 nm AuNPs at three concentration (0.1, 0.5, and 1 nmol particle per liter) for 24, 48, and 72 hours. Cell proliferation and viability were evaluated using trypan blue exclusion. TEM was used to image AuNPs internalized by the fibroblasts. To ascertain if AuNPs induce oxidative DNA damage, we analyzed the quantity of 8 hydroxydeoxyguanosine (8OHdG) which causes mutagenicity through G-C to T-A transversions resulting in 8OHdG-A mispaired bases.
Results: There were no visible alterations in the cell morphology between the treated and control (media only without AuNPs) groups at different time points, however, cell proliferation showed a significant difference in the total number of cells at 72 hours following AuNP treatment. AuNPs taken up by the fibroblasts showed up as dark dense clusters under TEM. They mostly gathered in clusters inside cellular vesicles. In some cases, scattered AuNPs were found in the cytosol. DNA damage analysis showed that 8OHdG/106dG value in 1 nmol particle concentration AuNP treated cells was significantly higher than the control, implying oxidative DNA damage when the cells were exposed to higher concentration of AuNPs.
Conclusions: AuNPs may induce some degree of cytotoxicity in human lung fibroblasts via inhibiting cell proliferation and oxidative damage.

1491 PULMONARY RESPONSE TO INTRATRACHEAL INSTILLATION OF FINE OR ULTRAFINE CARBON BLACK OR TITANIUM DIOXIDE: ROLE OF SURFACE AREA.

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Nanparticles are characterized by having a high surface area per mass. Therefore particle surface area may play an important role in determining the biological activity of nanoparticles. However problems arise because nanoparticles tend to agglomerate into μm sized particles when suspended in PBS.

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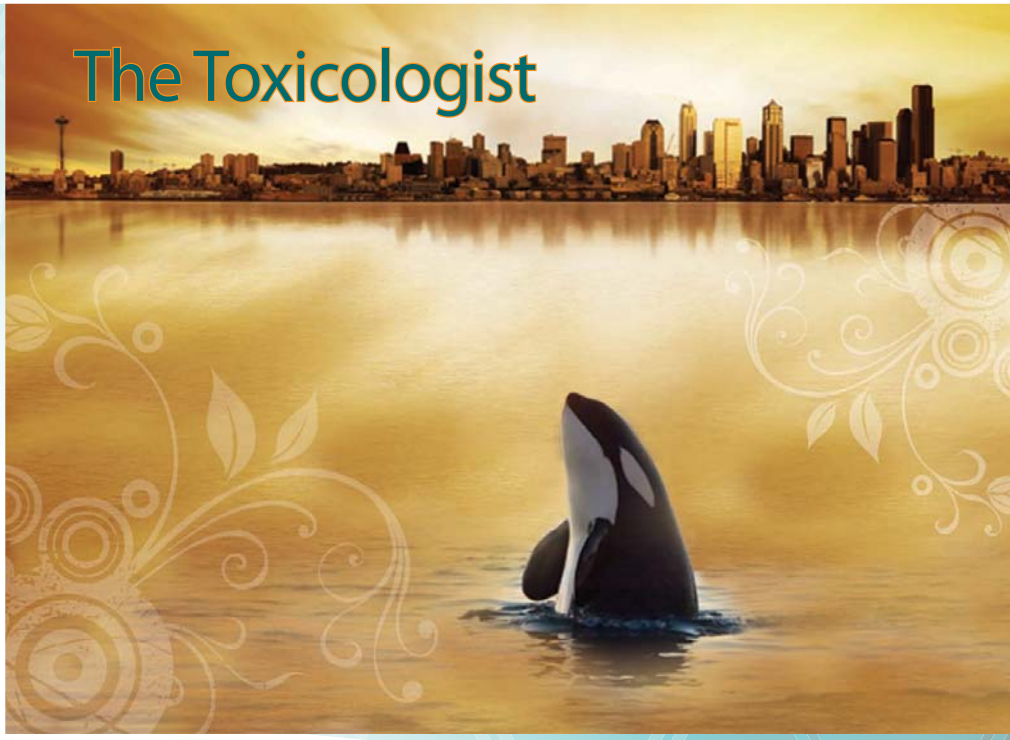
The Toxicologist

March 2008

The Toxicologist



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OXFORD UNIVERSITY PRESS ISSN 1096-6080 Volume 102, Number 1, March 2008