PS **253** **COMPARISON OF MIXTURE TOXICITY FOR MONO-, DI-, AND TRI-HALOGENATED ACETONITRILE COMBINATIONS.**

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Halogenated acetonitriles were tested alone and in binary mixtures using Microtox to assess relationships between chemical structure and combined effect. Derivatives tested included mono (iodo, bromo, chloro), di (dibromo, dichloro) and tri (trichloro) forms. Toxicity was assessed at 15, 30 and 45 minutes of exposure with seven concentrations and a control (each duplicated). Some agents were tested as sham mixtures (i.e., the agent with itself), thereby serving as positive controls for dose-addition. Data analysis gave EC50, concentration-response curve slopes and time-dependent toxicity (TDT) values for each agent and mixture. Data were fit to sigmoid curves using the four-parameter logistic function and evaluated against predicted curves for dose-addition. Sham combinations were dose-additive, as judged by EC50 additivity quotient (EC50-AQ) values of about 1.0. Slope AQ values were also near 1.0. Mixture toxicity for true combinations varied. Dose-addition was observed for each time-point with each of the three mono/mono combinations; wherein TDT and slope values were similar for each agent alone. Mixture toxicity of mono/di, mono/tri, di/di or di/tri combinations produced combined effects that varied, with some instances of apparent dose-addition and others that were clearly less-than dose-additive. Slopes of the concentration response curves and fit of the experimental mixture curves to predicted dose-addition curves at the EC25 and EC75 levels were useful in distinguishing between apparently dose-additive and dose-additive combinations versus when just EC50 data were considered. Slopes of the concentration-response curves should be considered in mixture toxicity testing as exemplified by results for the dibromo-dichloro and iodo-dichloro combinations.

PS **254** **HEATH-TREATED PM10 RETAIN A NON-VOLATILE COMPONENT RELATED PRO-OXIDATIVE POTENTIAL.**

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Current working hypothesis on the mechanisms mediating particulate matter (PM) toxicity resides on its capacity to induce oxidative stress on cells. PM metals and organics have the potential to cause it. However, further understanding of the major PM components responsible for driving the oxidative potential is needed. As part of the MILAGRO campaign in Mexico City we identified that PM10 pro-oxidative potential did not correlate with the PM components identified by EPR as having oxidative potential. To further understand the process, we expanded our study subjecting aliquots of the same PM samples to different temperatures (22, 50, 100, 150 and 175oC / 30 min) in order to evaporate volatile components. PM was sampled daily during March 2006 on cellulose membranes using High Vol samplers. Daily samples were pooled after being physically removed from the membranes. After temperature treatment, samples underwent Electron Paramagnetic Resonance (EPR) analysis (JEOL-JES TE-300) in the absence (CF 328 mT, 9.4 GHz, 10 mW, 2 min, amp 160) and presence (CF 335 mT, 9.4 GHz, 1 mW, 2 min, amp 100) of the .OH trapper DMPO (0.2 M), with and without 0.5 M H2O2. Paramagnetic species signal increased up to 20% after temperature treatment. EPR results in the presence of DMPO indicate that .OH generation by PM10 was only achieved in the presence of H2O2. Heat treatments did not modify the amount of generated .OH, remaining constant throughout the temperature treatment. These results indicate that pro-oxidative potential of PM10 is linked to non-volatile components in which metals may be playing a major role, since PM10 .OH generation is H2O2 dependant. Supported by MILAGRO-CAM and CONACyT 43138-M.

PS **255** **PHYTOTOXICITY OF METAL OXIDE NANOPARTICLES TO ARABIDOPSIS THALIANA.**

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The nanotechnology industry is projected to be worth approximately 2.6 trillion dollars by 2015. This estimation has escalated the concern over the potentially toxic effects of the nanoparticles, causing the recent advent of nanotoxicology. Thus far, only a few studies have considered the toxicity of nanoparticles to plants. Phytotoxicity of nanoparticles is important to fully understand the potential environmental impacts of manufactured nanomaterials. The present study reports on the effects of four metal oxide nanoparticles, aluminum oxide (nAl2O3), silicon dioxide (nSiO2), magnetite (nFe3O4), and zinc oxide (nZnO), on the development of Arabidopsis thaliana (Mouse-ear cress). Three toxicity indicators (seed germination, root elongation, and number of leaves) were quantified following exposure to each nanoparticle at three concentrations (400, 2,000 and 4,000 mg/L) for 18 days. Among these particles, nZnO was most phytotoxic, followed by nFe3O4, nSiO2, and nAl2O3, respectively. Consequently, nZnO was selected for further experimentations to determine the importance of particle size and zinc dissolution as toxicity determinants. Soluble zinc concentrations in nanoparticle suspensions were nearly 33-fold lower than the minimum inhibitory concentration of dissolved zinc salt (ZnCl2), indicating that zinc dissolution could not solely account for the observed toxicity. In addition, the inhibition of seed germination by ZnO depended on particle size with nanoparticles exerting higher toxicity than micron sized particles at equivalent concentrations. Overall, the present study demonstrates possible adverse effects of metal oxide nanomaterials on plants and calls for further research on the potential impacts of manufactured nanoparticles on agricultural and environmental systems.

PS **256** **INDUCTION OF ENDOTHELIAL DYSFUNCTION BY IRON OXIDE NANOPARTICLE EXPOSURE.**

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The correlation between nanoparticle exposure and cardiovascular diseases attracts much concern over the environmental-health effects of nanotechnology. Nanoparticle triggered endothelial dysfunction is hypothesized to be an important mechanism. In the present study, 22 nm-sized ferric oxide (Fe2O3) and 43 nm-sized ferriferrous oxide (Fe3O4) nanoparticles were selected to test this hypothesis and approach the biological pathways. Nanoparticles exposure to human aortic endothelial cells (HAECs) and monocyte-macrophages (U937 cells) in the separate-culture or co-culture system were applied to study the direct effects on HAECs and the indirect effects mediated by macrophage activation. The endothelial cell viability, membrane permeability and several crucial parameters involved in endothelial dysfunction were evaluated. Our results indicated exposure to iron oxide nanoparticles could induce HAECs vacuolization and cell death. Significant increase of HAECs membrane permeability and nitric oxide (NO) production were induced by activated-macrophages mediation. The monocyte adhesion to endothelial cells was enhanced associating with the up-regulation of intracellular cell adhesion molecule-1 (ICAM-1) and interleukin-8 (IL-8) expression. Iron oxide could be dissolved by macrophage phagocytosis. The overloading of phagocytosis activated macrophages and provoked oxidative stress. It is hypothesized that the intravascular iron oxide nanoparticles could affect the endothelial system by the nanoparticles, the released iron ions by phagocytosis, and the activated macrophage mediation. The induction of endothelial inflammation and dysfunction by iron oxide nanoparticles exposure were considered to be significant risk factors in atherogenesis.

PS **257** **CERIUM OXIDE, A DIESEL FUEL CATALYST, INDUCES PULMONARY FIBROSIS.**

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Cerium oxide (CeO_2) has been used as a diesel fuel catalyst in Europe to lower the mass of diesel exhaust particles (DEP). The potential pulmonary toxicity of CeO_2 nanoparticles, with or without DEP, has not been well characterized. Our previous studies have shown that CeO_2 induced pulmonary inflammation, cytotoxicity and air/capillary injury. The objective of this study was to determine the effects of CeO_2 on the induction of lung fibrosis. Male Sprague Dawley rats were exposed to CeO_2 (0.5 to 7 mg/kg) by a single intratracheal instillation. Alveolar cells were isolated via bronchoalveolar lavage (BAL) at 1, 10 and 28 days post-exposure. Exposure of rats to CeO_2 increased reactive oxygen species generation and apoptosis of alveolar macrophages (AM). Analysis of mRNA levels of several genes using real time RT-PCR showed that CeO_2 markedly increased suppressor of cytokine signaling-1 in BAL cells obtained at 1 day, which returned to the basal level at 28 days after exposure. In contrast, osteopontin (OPN) mRNA was significantly elevated in lung tissues in time-dependent manner. Exposure of rats to CeO_2 or DEP alone induced arginase-1 at 28 days post-exposure, suggesting switching of AM from an inflammatory, M1, to fibrotic, M2, subset that may lead to fibrosis. Increased amounts of

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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 49th Annual Meeting of the Society of Toxicology, held at the Salt Palace Convention Center, March 7–11, 2010.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 473.

The issue also contains a Key Word Index (by subject or chemical) of all the presentations, beginning on page 496.

The abstracts are reproduced as accepted by the Scientific Program Committee of the Society of Toxicology and appear in numerical sequence.

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phospholipids were demonstrated in the BAL fluid at 10 days post exposure in CeO_2-exposed lungs. Transmission electron microscopy further showed a significant amount of lamellar bodies in CeO_2- and CeO_2 plus DEP-exposed AM. At 28 days post-exposure, increased lung hydroxyproline content was evident in the CeO_2-exposed lung tissues. Histopathological analysis of CeO_2-exposed lungs showed large (alveolar sized) clumps of acellular material, but no granulomatous lesions at 28 days post-exposure. However, morphometric evidence, using sirius red staining for collagen, demonstrated that rats exposed to the combination of CeO_2 plus DEP exhibited significantly greater fibrosis than CeO_2 or DEP alone. These results show that CeO_2 exposure induces M2 AM differentiation, apoptosis, OPN expression, and lung fibrosis.

PS 258 DETECTION OF NANOPARTICLES IN THE PERFUSATE OF FLOW-THROUGH SKIN DIFFUSION CELLS.

K. E. Wiench[2], S. Schulte[2], S. Champ[2], G. Cox[2], N. Monteiro Riviere[1], R. Landsiedel[2] and W. Wohlleben[2]. [1]*Center for Chemical Toxicology Research and Pharmacokinetics, Raleigh, NC and* [2]*BASF SE, Ludwigshafen, Germany.*

Little is known about TiO2 and ZnO nanoparticles (NP) in UVB-damaged skin. In this study, weanling pigs were exposed to UVB (moderate erythema). The skin was dermatomed and placed in flow-through diffusion cells. The UV-radiated and the unexposed skin were treated with 4 sunscreen formulations: 10% coated rutile TiO2 in oil/water (o/w) and water/oil; 5% coated ZnO o/w; and 5% uncoated ZnO o/w. TiO2 had a particle size of 10x50 nm (agglomerates, ca.90-460 nm). ZnO had a mean size of 140 nm (ca.60-200 nm). Skin was perfused for 24h and processed for various microscopic techniques and for inductively coupled plasma – mass spectroscopy (ICP-MS). Perfusate was analyzed for Ti or Zn by ICP-MS. Ti or Zn was not detected in the perfusate samples independent of exposure, sunscreen formulation, or UVB radiation. Additionally, the perfusate samples were concentrated by analytical ultracentrifuge and analyzed by transmission electron microscopy / Energy Dispersive X-Ray Spectroscopy (TEM/EDXS). Inorganic NP was detected in some of the perfusate samples of the UVB exposed and the unexposed skin. Unique spherical particles were identified by EDXS as Si/O, nanosized NaCl crystals as well as Al, Mg, K and Ca containing NP. Spherical TiO2 NP having a different crystal structure than the tested form (by Field Emission Gun-TEM and High-Angle Annular Dark- Field Scanning Transmission Electron Microscopy) were detected. These NP in the perfusate was independent of exposure or non-exposure. SAXS (Small angle X-ray scattering) also confirmed that there was no correlation between these TiO2 NP and the TiO2 used in the sunscreen formulations. These results show that NP are ubiquitous even under strictly controlled conditions and it is extremely important to use suitable analytical methods to ensure correct data assessments.

PS 259 LECTINS MODULATE MULTI-WALLED CARBON NANOTUBES CELLULAR UPTAKE IN HUMAN EPIDERMAL KERATINOCYTES.

L. W. Zhang and N. A. Monteiro-Riviere. *Center for Chemical Toxicology Research and Pharmacokinetics, North Carolina State University, Raleigh, NC.*

The development of nanomaterials for biomedical applications has attracted a great deal of attention. Carbon nanotubes may interact and cross cell membranes and serve as potential carriers for drug delivery studies. The reflection mode in the confocal laser scanning microscope was used to image multi-walled carbon nanotubes (MWCNT) uptake in human neonatal epidermal keratinocytes (HEK) stained with the cytoskeleton protein F-actin. Scanning electron microscopy depicted tight binding of MWCNT on the plasma membrane of HEK while some MWCNT were located in the cell. Since keratinocytes normally engulf melanosomes, we hypothesized that the melanocyte transfer pathway could be a potential route of entry into keratinocytes. Lectins which are inhibitors of melanosome transfer were used to study the uptake by MWCNT in keratinocytes, to see if they played a role in reducing the cellular uptake of MWCNT in HEK. Three different lectins, *Pisum sativum* (PS), *Lycopersicon esculentum* (LE), and *Tetragonolobus purpureas* (TP) were used as a cocktail. The maximal concentrations of lectins that would be non-toxic to the HEK was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) cell viability assay. These studies confirmed that lectin cocktails (PS 5μg/ml, LE 25μg/ml and TP 25μg/ml) decreased MWCNT interaction with the cell surface and uptake. F-actin, a cytoskeleton protein, was used to visualize how the MWCNT interacted with the cytoskeleton in the cells. MWCNT were found to traverse through the cells' cytoskeleton and the plasma membrane into adjacent cells. (Supported by Dermal Toxicology Specialty Section of the National Society of Toxicology Battelle Student Research Award)

PS 260 TITANIUM DIOXIDE EXPOSURE INHIBITS ALLERGIC ASTHMA RESPONSE.

K. Savolainen, L. Pylkkänen, E. Rossi, H. Wolff and A. Harri. *Finnish Institute of Occupational Health, Helsinki, Finland.*

Effects of airways exposure to different materials may depend on the allergic status of the exposed individuals and thus we have investigated the pulmonary inflammatory responses against two different sized of titanium dioxides on the allergic asthma mouse model.

Mice were allergen sensitized by administering ovalbumin-aluminum suspension intraperitoneally on day 1 and 10 and exposed either to nanosized or coarse TiO2 aerosol (10 mg/m3) for two hours on days 18-21 of the 22 day experiment. On days 19-21 the mice were given 50 micrograms of ovalbumin intranasally to elicit asthma. On day 22 the bronchial responsiveness to methacholine was determined by whole body plethysmography, after which the mice were sacrificed. Inflammatory cell infiltration was characterized from bronchoalveolar lavage fluid, and expression of cytokines and chemokines relevant to inflammation in the lung tissue was assessed by real-time PCR.

Interestingly, asthmatic mice showed remarkable suppression of most mediators and signs of allergic asthma when exposed to both nanosized and coarse TiO2. The levels of leucocytes, cytokines, chemokines and antibodies relevant in allergic asthma as well as airway hyperresponsiveness were all decreased or even returned to a level normal in healthy mice. The results show that repeated airway exposure to TiO2 particles modulates the airway inflammation depending on the allergic status of the exposed mice.

Our results suggest that that there is a systemic inhibition caused by titanium dioxide inhalation that prevents symptoms of allergic asthma. However, on the other hand it is of notice, that exposure can pose other dangers. It can be therefore concluded that in assessing health implications of particle exposure in human populations it is remarkably important to consider the heterogeneity of the state of health of individuals in that population.

PS 261 ACELLULAR ASSESSMENTS OF ENGINEERED-MANUFACTURED NANOPARTICLE BIOLOGICAL SURFACE REACTIVITY.

K. Dreher[1], D. Dunlap[2] and T. R. Nurkiewicz[3]. [1]*NHEERL, U.S. EPA, Research Triangle Park, NC,* [2]*North Carolina State University, Raleigh, NC and* [3]*Center for Cardiovascular and Respiratory Sciences, West Virginia University, Morgantown, WV.*

It is critical to assess the surface properties and reactivity of engineered-manufactured nanoparticles (NPs) as these will influence their interactions with biological systems, biokinetics and toxicity. We examined the physicochemical properties and surface reactivity of metal oxide NPs (TiO2, CeO2, and SiO2) in several acellular assays to examine their "reactive" metal content, nitrosylation potential and ability to deplete nitric oxide (NO). The thiobarbituric acid substance assay (TBARS) was used to examine NP reactive metal content. Newly developed assays assessed NP nitrosylation and NO depletion properties. TBARS and nitrosylation assays were modified to determine photocatalytic properties of NPs. Nanoscale (<100 nm) NPs displayed the greatest activity compared to non-nanoscale (>100 nm) NPs in all assays where activity was detected. At equivalent concentrations SiO2 and CeO2 NPs displayed no endogenous or photocatalytic activity when examined in either the TBARS or nitrosylation assay. In contrast, at equivalent concentrations TiO2 NPs displayed low levels of detectable endogenous and very high levels of photocatalytic activity in the TBARS assay. TiO2 NP TBARS activity did not correlate with size or surface area as maximal activities were observed in the 25–32 nm size range and were influenced by crystalline structure. NPs displayed variable NO depletion potential (CeO2>TiO2>SiO2). TiO2 NO depletion did not correlate with size or surface area as maximal activities were observed in the 25–32 nm size range and were influenced by crystalline structure. In contrast CeO2 NO depletion did correlate with size and surface area. These results demonstrate that the complex biologic effects of NP on oxidative stress, protein modification, cellular function and toxicity preclude a simple prediction based on size and surface area alone. (This abstract does not reflect EPA Policy)

Support: NIH RO1-ES015022 and RC1-ES018274 (TRN)

PS 262 TITANIUM DIOXIDE NANOPARTICLES CAUSE GENOTOXICITY IN HUMAN LUNG EPITHELIAL CELLS.

R. Y. Prasad[1], K. Wallace[2], A. H. Tennant[2], K. T. Kitchin[2], A. D. Kligerman[2], J. G. Muñiz Ortiz[2] and C. F. Blackman[2]. [1]*Student Contractor, Integrated Systems Toxicology Division, U.S. EPA, Research Triangle Park, NC and* [2]*Integrated Systems Toxicology Division, U.S. EPA, Research Triangle Park, NC.*

The use of engineered nanoparticles in consumer products is steadily increasing. However, the health effects of exposure to these nanoparticles are not thoroughly understood. This study investigated the genotoxicity of six titanium dioxide and