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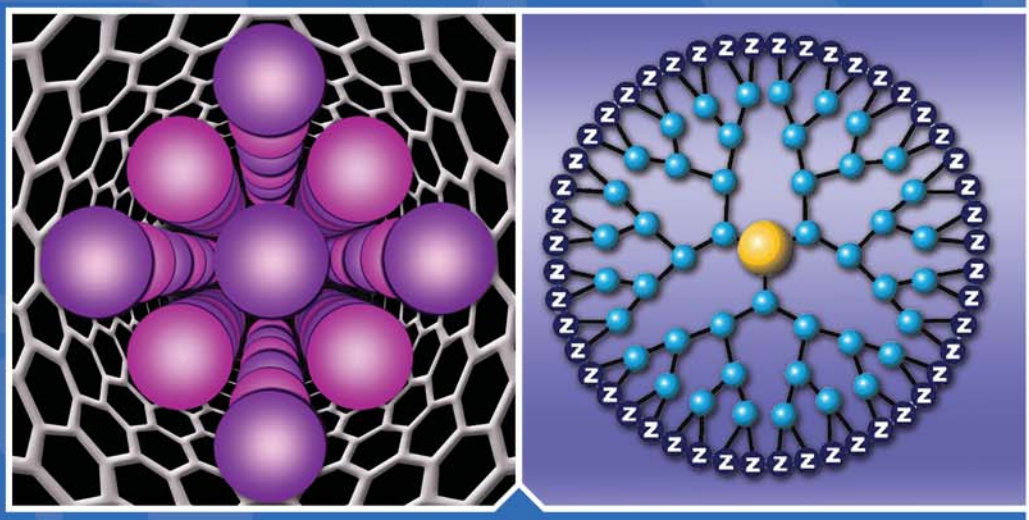

Office of Research and Development
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Metals, Metal Oxides

Fate, Transformation, and Toxicity of Manufactured Nanomaterials in Drinking Water

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Objective: Although the current market for nanomaterials is small and their concentration may not be high enough in the environment to cause human health or environmental problems, this market is increasing rapidly, and the discharge of nanomaterials to the environment in the near future could be significant as manufacturing costs decrease and new applications are discovered. The accumulation of nanomaterials in cells may have significant environmental and human impacts. However, at present, very little is known about the fate, transport, transformation, and toxicity of these man-made nanomaterials in the environment. The objectives of this project are to: (1) characterize the fundamental properties of nanomaterials in aquatic environments; (2) examine the interactions between nanomaterials and toxic organic pollutants and pathogens (viruses); (3) evaluate the removal efficiency of nanomaterials by drinking water unit processes; and (4) test the toxicity of nanomaterials in drinking water using a cell culture model system of the epithelium. This study considers the physical, chemical, and biological implications of nanomaterial fate and toxicity in systems that will provide insight into the potential for nanomaterials to be present and to pose health concerns in finished drinking water.

Approach: A multidisciplinary approach is proposed that includes experiments to identify fundamental uniqueness of nine nanomaterial properties and toxicity and quite applied experiments aimed directly at understanding the fate and reactions involving nanomaterials in drinking water treatment plants. Advanced nanomaterial characterization techniques will be employed to determine the size distribution, concentration, and zeta potential of nanomaterials in buffered distilled water and model waters representative of raw drinking water supplies (anions, cations, natural organic matter [NOM]). Adsorption of dissolved pollutants (anions, metals, range of synthetic organic chemicals) and NOM are proposed to quantify the potential for nanomaterials to transport such compounds and be transformed by the compounds (e.g., via aggregation, change in zeta potential). Coagulation processes will be studied by compressing the electric double layer of nanomaterials and exposing nanomaterials to alum coagulations, using mono- and heterodisperse solutions; comparable filtration work also will be conducted. Adsorption of virus onto nanomaterials and subsequent disinfectant shielding will be studied. Toxicity screening will include toxicity of nanomaterials on several cell lines selected to mimic oral ingestion routes in drinking water.

Expected Results: This project will provide fundamental information about the fate, transport, and transformation of nanomaterials in drinking water resources and the first evidence that such nanomaterials can or cannot be removed by conventional drinking water treatment processes. An improved assessment will be developed for the potential exposure risks of nanomaterials in drinking water. This research would ultimately provide essential information that would support policy and decisionmaking regarding handling, disposal, and management of nanoscale materials in commerce, manufacturing, and the environment.

EPA Grant Number: R831713

Pulmonary and Systemic Inhalation Toxicity of Multiwalled Carbon Nanotubes

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Inhalation of multiwalled carbon nanotubes (MWCNTs) at particle concentrations ranging 0.3–5 mg/m^3 did not result in significant lung inflammation or tissue damage, but caused systemic immune function alterations. C57BL/6 adult (10-12 week) male mice were exposed by whole-body inhalation to control air or 0.3, 1, or 5 mg/m^3 respirable aggregates of MWCNTs for 7 or 14 days (6 hours/day). Histopathology of lungs from exposed animals showed alveolar macrophages containing black particles; however, there was no inflammation or tissue damage observed. Bronchial alveolar lavage fluid also demonstrated particle-laden macrophages; however, white blood cell counts were not increased compared to controls. MWCNT exposures to 0.3 mg/m^3 and higher particle concentrations caused nonmonotonic systemic immunosuppression after 14 days, but not after 7 days. Immunosuppression was characterized by reduced T-cell-dependent antibody response to sheep erythrocytes, as well as by T-cell proliferative ability in the presence of the mitogen, Concanavalin A (Con A). Assessment of nonspecific natural killer (NK) cell activity showed that animals exposed to 1 mg/m^3 MWCNTs had decreased NK cell function. Gene expression analysis of selected cytokines and an indicator of oxidative stress were assessed in lung tissue and spleen. No changes in gene expression were observed in lung; however, interleukin 10 (IL-10) and NAD(P)H oxidoreductase 1 (NQO1) mRNA levels were increased in the spleen.

EPA Grant Number: R832527

Pharmacokinetics and Biodistribution of Quantum Dot Nanoparticles in Isolated Perfused Skin

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The disposition and pharmacokinetics of nanoparticles in tissues are crucial parameters for targeting nanotechnology-based drug delivery systems as well as for defining their toxicological profile. Quantum dots (QD), nanomaterials that naturally fluorescence, can be synthesized with varying surface coatings that modulate disposition and are amenable to localization in skin and other tissues due to intense fluorescence. QD were synthesized with a 8.40 nm x 5.78 nm CdSe core and either polyethylene glycol (PEG) or COOH coatings. These QD621 have a maximum emission wavelength of 621 nm and a hydrodynamic size (in water) of approximately 37 nm. Flow-through diffusion cells were used to assess QD penetration through porcine skin, along with laser scanning confocal microscopy (LSCM). The isolated perfused porcine skin flap (IPPSF) was used to determine whether intra-arterially perfused QD would distribute to the skin. QD were mixed with 300 mL of media and were intra-arterially infused into the IPPSF (6.67 nM, 3.33 nM, 1.67 nM, or 0.83 nM) for 4 hours (dose phase), and then QD media was replaced with fresh media and the IPPSF was perfused for an additional 4 hours (washout phase). Upon termination of the perfusion, the IPPSF was cut into 6 segments, flash-frozen in liquid nitrogen, cryosectioned at 25 μm, and imaged by LSCM. The arterial and venous perfusate was sampled and the fluorescence quantitated. Flow-through diffusion cells showed penetration of QD621 only in the upper stratum corneum layers of skin. This is in contrast to studies with QD565 and QD655 that showed slight coating-dependent epidermal penetration. In the QD621 infusion study, COOH-coated QD had greater tissue extraction than PEG. Images indicate aggregation of infused QD in the skin vasculature. Transmission electron microscopy localized QD621 within the capillary walls. A pharmacokinetic model of arterial-venous extraction and tissue biodistribution of QD was developed based on a model previously used to quantitate platinum distribution in the same experimental system. Significant arterial-venous QD extraction was observed at all doses, with COOH QD showing greater predicted tissue deposition, an agreement in line with the confocal studies above. A unique kinetic finding was periodicity (approximately 90 minutes) in arterial extraction, an observation not seen after chemical infusions. These data begin to define nanomaterial characteristics that correlate to tissue uptake and persistence. They are important for risk assessment and drug delivery, because they suggest that QD not specifically targeted for medical applications can biodistribute to tissues, have unique pharmacokinetic patterns of arterial extraction, and potentially may cause adverse effects.

EPA Grant Number: R831715

Metal Nanoparticle Tissue Distribution Following *In Vivo* Exposures

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Manipulation of the physicochemical properties of materials at the nanoscale has the potential to revolutionize electronic, diagnostic, and therapeutic applications. Because of the potential large-scale use of nanomaterials, it is important to determine if there is any unique toxicity of the nanoscale materials as compared to the bulk. As has been hypothesized for nanosized ambient air, or ultrafine particles, nanoparticles (NP) may evade particle clearance mechanisms at the site of exposure, thus potentially coming into contact with epithelial and endothelial cells and translocating to sites distant from original deposition. It also is possible that inflammation and oxidant stress will occur as a result of unique NP properties or from prolonged retention. In the last year of this project, we have focused on the biodistribution and fate of engineered NP that were administered via the respiratory tract or systemically. The first 2 years of the project were focused on detailed physicochemical characterizations of the NP systems we used to test our hypotheses and on the *in vitro* uptake and effects of NP, particularly nanosized Pt (flowers, multipods, flower spheres, and pod spheres; 11-35 nm; 1-27 m^2/g). Acellular reactivity assays, as well as *in vitro* and *in vivo* assessments of toxicity and inflammatory potential, revealed that the Pt NP are relatively nontoxic, with activity similar to that of nanosized TiO_2. We quantitated the uptake of the Pt shapes by cultured endothelial cells and found that the particles with larger surface area per mass (Pt flowers) were taken up to a greater degree than those with smaller surface area (Pt multipods). Results from preliminary *in vivo* exposures in rats also showed that Pt flowers were retained in the lungs to a greater extent than Pt multipods, although neither particle type induced severe inflammation. We also found that a significant fraction (~80%) of the instilled dose was cleared from the lungs within the first 24 hours following exposure. To investigate more specifically the impact of particle surface on tissue distribution, we exposed rats to quantum dots (QDs; CdSe-ZnS core-shell crystals with polymer cap and biomolecule coating) with three different surface functionalizations (PEG, PEGamine, carboxylic acid) via intratracheal microspray (ITM) and intravenous (IV) exposures. We found that QDs delivered via ITM did not induce a severe lung inflammatory response 24 hours after exposure (maximum of 3.7% neutrophils in lavage fluid, carboxyl QDs) and that they did not, for the most part, translocate out of the lungs. The PEGamine- and carboxyl-coated QDs were found in the lung-associated lymphoid tissue (Cd signal), but Cd was not detected in any of the other tissues we examined. Following IV exposure, the QD surface characteristics significantly impacted tissue localization. For example, PEG-coated QDs had the highest retention in most tissues; however, they did not accumulate in the bone marrow, whereas both the PEGamine- and carboxyl-coated QDs did. Tissues from exposed rats are currently being examined using fluorescence microscopy to identify the cell types that might take up QDs in tissues where significantly elevated Cd signals were found. We are currently performing a more detailed biokinetics study (1 hour, 24 hours, 7 days postexposure) of QD tissue distribution following ITM and IV exposures and collecting excreta so that we can more fully account for the delivered dose of material. These studies, through comparisons with other metal NP, are helping to define the biodistribution of nanomaterials as a function of their physicochemical characteristics and also to establish NP-related effects following *in vitro* and *in vivo* exposures.

EPA Grant Number: R831722

The Bioavailability, Toxicity, and Trophic Transfer of Manufactured ZnO Nanoparticles: A View From the Bottom

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Objectives: The overall objectives of this research project are to: (1) evaluate the bioavailability and toxicity of manufactured nanoparticles (ZnO) as a function of particle size to the model soil bacteria, *Burkholderia cepacia,* and the model detritivore, *C. elegans,* as referenced against aqueous Zn^{2+}; (2) evaluate the ability of manufactured ZnO nanoparticles to be transferred from one trophic level to the next, as assessed in the simple food chain consisting of preexposed *B. cepacia* and *C. elegans*; and (3) evaluate the synergistic or antagonistic effects of manufactured ZnO nanoparticles on the toxicity of Cu^{2+} to *B. cepacia* and *C. elegans*. These three overall objectives will be approached in the context of the following four hypotheses:

- Hypothesis 1: The bioavailability and toxicity of manufactured ZnO nanoparticles increases with decreasing particle size (i.e., 6 nm versus 80 nm).
- Hypothesis 2: The toxicity of ZnO nanoparticles to *B. cepacia* and *C. elegans* is lower than an equivalent concentration of dissolved Zn^{2+}.
- Hypothesis 3: The bioavailability and toxicity of ZnO nanoparticles introduced via trophic transfer differs from direct exposure.
- Hypothesis 4: ZnO nanoparticles alter the bioavailability and toxicity of dissolved metals.

Approach: We will study the influence of particle size of ZnO nanoparticles, (i.e., 3 nm versus 80 nm) on bioavailability and toxicity (lethal and sublethal effects) and will compare these results with exposure to an equivalent concentration of aqueous Zn^{2+}. Additionally, we will examine the effect of nanoparticles on the toxicity of a dissolved constituent (Cu^{2+}). We will employ optical and fluorescent microscopy, element-specific, synchrotron-based microspectroscopy, and hyphenated separations-ICPMS techniques to determine the distribution of nanoparticles within each organism and potential transformations of nanoparticles. Additionally, we will employ a transgenically modified strain of *C. elegans* in which we have incorporated a metal-specific promoter (metallothionein-2 [mtl-2]) that turns on expression of green fluorescent protein (GFP) in the presence of bioavailable metals. We expect that the nanoparticles will not switch on the GFP promoter, but transformations (dissolution) of the nanoparticles that release the free metal will induce GFP expression. Additionally, this transgenic strain will be used to study the effect of the bioavailability of Cu^{2+} in the presence of ZnO nanoparticles and the potential that bioavailability will be lowered as indicated by lower GFP expression. These observations will be coupled with measurements of lethal and sublethal responses for *C. elegans* exposed directly and indirectly from grazing on preexposed *B. cepacia*, including behavior and reproduction. We speculate that *C. elegans* will bioaccumulate greater quantities of ZnO nanoparticles when feeding on preexposed *B. cepacia* compared to direct exposure as a result of the likelihood that intracellular ZnO nanoparticles will be surface-modified by biocompatible molecules (e.g., peptides, proteins, other intracellular ligands) in *B. cepacia*.

Expected Results: These studies will provide among the first data on the bioavailability and toxicity of a widely used nanoparticle/nanocomposite (ZnO) to a model bacteria and detritivore and the first data available on potential for manufactured nanoparticles to be transferred through the food chain. The general lack of information on the bioavailability and toxicity of manufactured nanoparticles to microorganisms and higher organisms and on the ability of manufactured nanoparticles to be transferred from prey to predators leads to a number of very basic questions that will need to be resolved to ensure that the potential human health and ecological risks associated with the widespread use and disposal of manufactured nanoparticles are properly evaluated. This project will provide critical information needed to begin to bridge these knowledge gaps.

EPA Grant Number: R832530

Biochemical, Molecular, and Cellular Responses of Zebrafish Exposed to Metallic Nanoparticles

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The goals of this research project are to: (1) determine if metallic nanoparticles produce toxicity that is distinct from that of soluble forms of the metal in zebrafish; and (2) determine how physical properties of particles are related to toxicity. To this end, we have examined the behavior of metal particles in aqueous environments over time, with respect to particle aggregation, surface charge, and dissolution. All particles tested exhibited aggregation in aqueous suspensions. Mean particle size by volume increased to 20 microns 48 hours after addition of 50-nm copper nanoparticles to water. Despite their small volume contribution, large numbers of small particles remained in suspension for the duration of the experiment. Under these conditions, little or no change in zeta potential occured. Aluminum, nickel, and silver nanoparticles produced little or no lethality in zebrafish exposed to concentrations up to 10 mg/L for 48 hours. However, exposure to aluminum nanoparticles produced changes in gill structure and function as well as changes in gene expression. Unlike these metals, exposure to copper nanoparticles produced lethality in zebrafish within 48 hours. Copper nanoparticles were less acutely toxic to adult female zebrafish than copper sulfate, with a 48-hour LC50 of 1.5 mg/L for nanocopper versus 0.25 mg/L for copper sulfate. The lethal effects of copper nanoparticle exposure appeared to be mediated at least in part by the particles and not solely by dissolution. In tanks treated with 1.5 mg/L copper particles, only 0.1 mg/L of dissolved copper was present at 48 hours, which is equivalent to a concentration of copper sulfate producing 15 percent mortality. This conclusion also was supported by differences in biochemical and molecular changes following exposure to the two forms of copper. Serum BUN and ALT levels, gene expression patterns in liver, and liver histopathology showed similar minimal responses to both forms of copper. Both forms of copper also produced injury to the gill epithelium; however, the observed gene expression responses were markedly different in gill samples, indicating that the particles induced a different transcriptome level response than did copper sulfate. We, therefore, conclude that copper nanoparticles exert a toxic effect on zebrafish gill that is not solely the result of dissolution of the particles.

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Acute and Developmental Toxicity of Metal Oxide Nanoparticles to Fish and Frogs

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Objectives: The objectives of this research are to determine the environmental hazard associated with selected metal oxide nanoparticles (Fe_2O_3, ZnO, CuO, and TiO_2) in terms of acute and chronic toxicity to fathead minnows (*Pimephase promelas*) and the African clawed frog (*Xenopus laevis*). The hypotheses are that nanoparticle exposure will affect the survival, growth, development, egg hatchability, and metamorphosis of these organisms in a dose-dependent fashion, and differences in relative toxicity (LC50, EC50, NOEC, LOEC) of these nanoparticles will coincide with the relative toxicity of their soluble salts or oxides.

Approach: Fathead minnows and frogs will be exposed to metal oxide nanoparticles during 96-hour acute toxicity and developmental toxicity tests. Chronic tests will include 28-day early life stage tests (starting within 24 to postfertilization) for minnows and 10-week exposures (hatch until metamorphosis completion) for *X. laevis*. Endpoints will include survival, growth, percent hatch, developmental abnormalities, and rate of metamorphosis (for *X. laevis*). Acute toxicity (growth, survival) endpoints will be reported as LC50s, and chronic toxicity endpoints will be reported as EC50s, NOECs, and LOECs. Nanoparticles will be kept in suspension in the water using aeration- or peristaltic pump-induced water currents (i.e., minimizing settling of nanoparticles). Mixing of aged and fresh nanoparticles in test solutions will be minimized using flow-through systems. Physiochemical characterization of nanoparticles before and during tests will be conducted by atomic force and electron microscopic methods. Metal concentrations will be monitored in water and tissues by means of atomic absorption spectrophotometry. Nanoparticles will be synthesized chemically at Clemson University.

Expected Results: It is expected that the nanoparticles will increase mortality and developmental abnormalities in fish and frogs and decrease growth rates, rates of metamorphosis, and hatchability. Calculation of LC50s and EC50s for acute and developmental toxicity is of benefit because these chemicals have the potential for widespread release into aquatic environments, either due to large-scale manufacture or use or to applications in decontamination of groundwater and waste streams. However, little, if anything, is known about their potential hazard in aquatic environments. The LC50s and EC50s would allow ecological risk assessment of these particles at an early stage in the development of this technology. It should be noted that, even if none of these nanoparticles show any affect on minnow or frog larvae, this would still be useful information.

EPA Grant Number: R832842

Mechanistic Dosimetry Models of Nanomaterial Deposition in the Respiratory Tract

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Objective: Accurate health risk assessments of inhalation exposure to nanomaterials will require dosimetry models that account for interspecies differences in dose delivered to the respiratory tract. Mechanistic models offer the advantage to interspecies extrapolation that physicochemical properties of particles and species differences in ventilation, airway architecture, and physiological parameters can be incorporated explicitly to describe inhaled dose. The objective of this research is to extend existing, verified mechanistic models of particle deposition in the respiratory tract of rats and humans both to cover the range of size for nanoparticles and nanotubes. Deposition mechanisms are described based on first principles and semi-empirically as required. Semi-empirical models of penetration from the upper respiratory tract (URT) also can be used to describe regional deposition fraction in the URT and could be extended to localized modeling. The approach includes model verification with experimental data obtained both in human and rat casts of the upper respiratory tract as well as *in vivo* studies of respiratory tract deposition.

Approach: Manufactured nanoparticles and nanotubes will be obtained from manufacturers and generated in our laboratories. Deposition of nanomaterial will be measured in nasal casts of humans and rats. These data will allow calculation of the fraction of inhaled material that passes through the URT and enters the lower respiratory tract (LRT). Next, existing models of LRT deposition will be extended to include mechanisms for nanomaterial. For nanoparticles, existing models for fine and coarse particles will be extended by accounting for the mechanisms of axial diffusion and mixing. This will address the previous inadequate treatment of dispersive effects in the existing models that has limited their applicability to nanosized particles. For nanotubes, deposition depends on nanotube orientation in the air. Net orientation of a cloud of nanotubes entering each airway will be found to calculate their deposition. A software package with a graphical-user interface will be developed to provide rapid computational capabilities to run simulations based on these models. A series of nose-only exposure events in Long-Evans rats will be conducted to measure regional and lobar deposition of nanoparticles in the respiratory tract. Deposition models will be verified in rats by comparing deposition predictions against measurements from nose-only exposures, and in humans by comparing the model predictions against available data in the literature.

Expected Results: This effort will result in mechanistic dosimetry models to predict the localized deposition of inhaled nanomaterial in the respiratory tract of rats and humans. Specific products include:

- Deposition measurements of nanosized particles in casts of human and rat nasal URT airways
- Semi-empirical relationships to predict nanomaterial deposition in the URT airways
- Respiratory tract deposition models of nanoparticles and nanotubes in humans and rats
- Measurements of regional and lobar deposition of nanomaterial in the heads and lungs of rats
- A user-friendly software package to implement models and provide rapid simulation capability.

EPA Grant Number: R832531

Nanostructured Materials for Environmental Decontamination of Chlorinated Compounds

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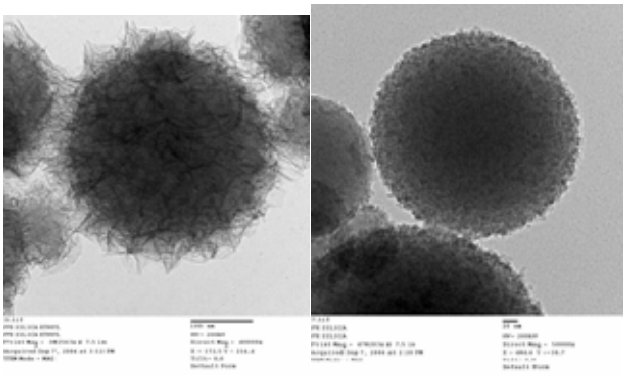
This research project is directed towards the development of novel mesoporous materials that act as supports for zerovalent iron nanoparticles used in the breakdown of chlorinated compounds. Halogenated organic compounds, such as chlorinated aromatics, chlorinated aliphatics, and polychlorinated biphenyls, are typical of dense nonaqueous phase liquids (DNAPLs) that are prevalent at contaminant sites. In recent years, the use of zerovalent iron has represented a promising and innovative approach to the destruction of these compounds. Of particular interest is the number of publications recently that describe the use of nanoparticles of iron (Fe) in remediation through hydrodechlorination. The enormous surface area of nanoparticles leads to enhanced efficiencies. Additionally, the colloidal nature of Fe nanoparticles indicates that these materials may be pumped to contaminated sites. Alternatively "funnel and gate" treatment systems may be devised, where porous barriers of iron particles are constructed in the path-contaminated groundwater plumes.

Due to the high surface energy of nanoparticles, iron nanoparticles tend to aggregate, leading to larger units that do not maintain colloidal stability. Although Fe nanoparticles that exceed 10-15 nm exhibit ferromagnetism, this also leads to aggregation and inefficient transport. Finally, Fe is hard to functionalize with organic compounds to attempt to maintain stability in aqueous or in organic systems. Our technical approach combines the simplicity and affordability of the sol-gel processing techniques for ceramic synthesis with the efficiency and spontaneity of surfactant/silica cooperative assembly to manufacture nanostructured decontamination materials. We use a simple aerosol processing technique to encapsulate Fe nanoparticles in silica microparticles that can be easily functionalized, leading to facile transport to trichloroethylene (TCE) interfaces and partitioning at the TCE-water interface. Sample morphologies of such particles are shown below. Our results indicate the following: (1) functionalized composite particles significantly adsorb TCE; (2) composite particles are effective in TCE decontamination; (3) composite particles partition to the TCE-water interface; and (4) composite particles have optimal size characteristics to be effective in transport through sediments. Representative particles are shown below.



EPA Grant Number: GR832374

Responses of Lung Cells to Metals in Manufactured Nanoparticles

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Objective: This research project is based on the hypothesis that transition metals in particles induce proinflammatory signaling and cell damage through the production of reactive oxygen species. Established cell culture models and toxicology assays will be applied to the analysis of manufactured nanomaterials. Based on the literature and our own data, we expect that the small physical size and high surface area of nanoparticles (d < 30 nm) will increase cellular uptake and increase induction of proinflammatory signaling, compared to larger particles with the same elemental composition. *In vitro* studies with human and rat lung cells will evaluate the effects of manufactured nanoparticles in the as-sold condition, and the same materials after the particles have been subjected to surface modification simulating fire and wastewater treatment conditions. The emphasis will be on lower cost nanomaterials that are sold in powder or liquid suspension form, because these materials are expected to be produced and ultimately released in the largest amount.

Approach: A phased approach will be used to maximize useful results within the budget. In the first phase, low-cost assays will be used to screen a wide range of samples with sufficient replicates for statistical power. This phase will emphasize measurement of cytotoxicity, induction of the proinflammatory cytokine IL-6, and dissolution rate in simulated lung fluid. Industrial collaborators will assist in prioritizing materials for testing and in providing chemically similar materials of various sizes and grades. Materials selected in the screening phase will be used for more detailed, mechanistic studies. The second phase will test selected materials for particle uptake by the cells, for the induction of additional cytokines, and for the effect of antioxidants. Phase two physical characterization will include electron microscopy, BET surface area, zeta potential, and trace element analysis. In the third phase, the most inflammatory and most benign nanomaterials will be used in hypothesis-based toxicology experiments to evaluate plausible mechanisms by which the particles induce specific responses in cells. Cell culture toxicology studies with BEAS-2B cells, an immortalized human lung epithelial cell line, are emphasized and consistent with the goal of refining, reducing, and replacing animal use. However, it is necessary to establish the relevance of cell culture data to whole animals and to human health. Experiments using normal macrophages and normal epithelial cells that are freshly harvested from rats will be conducted to test the ability of the cell culture assays to predict the induction of inflammation by specific nanomaterials.

Expected Results: The screening phase will provide new data on a range of commercially available nanoparticles, using a consistent set of physical and cell culture assays to facilitate comparisons between materials. The surface modification studies will contribute to understanding the environmental fate of nanoparticles by evaluating whether the treatments enhance or decrease the biological effects of specific nanomaterials. The evaluation of plausable mechanisms and the experiments with freshly isolated rat airway cells will provide a transition between cell culture studies, inhalation studies, and extrapolation to sensitive human populations.

EPA Grant Number: R831723

A Toxicogenomics Approach for Assessing the Safety of Single-Walled Carbon Nanotubes in Human Skin and Lung Cells

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High-throughput biotechnologies were used to screen for toxicity of nanomaterials in this combined toxicogenomics and systems biology approach. Primary human epidermal keratinocytes and primary human bronchial epithelial cells were exposed *in vitro* for 24 hours to single-walled carbon nanotubes and other nano- and low-micron-scale particulate substances. RNA isolated from the cell pellets was copied, labeled, and hybridized onto gene expression microarrays containing between 10,000 and 20,000 human genes. A complete comparison between these two cell systems, using a four-tiered bioinformatics approach, was performed. Statistical analysis showed that the triplicate arrays run for each biological sample was very reproducible. Hierarchical agglomerative clustering showed that the greatest variation between gene expression profiles was between the two cell systems, regardless of nanomaterial exposure. Potential biomarkers were identified, and several correlated with previous literature references. Pathway analysis showed that the active pathways in both cellular systems were genes and proteins involved in membrane integrity and remodeling.

NSF Grant Number: 0536679

Microbial Impacts of Engineered Nanoparticles

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Fullerenes compose a class of nanomaterials that show potential for imminent medical, industrial, and technological applications. One model fullerene, C_{60}, is insolube in water, but will form a suspension termed nC_{60} upon extended exposure to water or after introduction to water via a solvent. The microbial impacts of nC_{60} are analyzed using the bacteria *Escherichia coli* and *Bacillus subtilis* as indicator species of both environmental impact and potential toxicity to higher level organisms. nC_{60} displayed strong antimicrobial properties against both bacteria, with a number of factors, such as salt concentration and particle size, mitigating toxicity. Several eukaryotic studies have implicated reactive oxygen species (ROS) as the mediators of toxicity. However, ROS may not be the only factor responsible for killing prokaryotic cells, as the antibacterial activity of nC_{60} persists in the absence of light and oxygen, challenging the feasibility of photocatalytic ROS formation. Other research on fullerenes suggests that they exert their antibacterial effect via direct damage to the cell membranes. This research project explores three possible mechanisms for the antibacterial activity of nC_{60}. It could: (1) physically disrupt the cell membrane; (2) generate ROS; or (3) exert ROS-independent oxidative stress. Results from flow cytometry analysis and other analytical techniques point to nC_{60} acting as a direct oxidant, possibly requiring direct contact with the cell. Defining the antibacterial mechanism allows the manipulation of the antibacterial activity for both disinfection applications and mitigation of undesired environmental impacts.

EPA Grant Number: R832534

An Integrated Approach Toward Understanding the Inflammatory Response of Mice to Commercially Manufactured CuO/Cu, Fe_2O_3/Fe, and TiO_2 Nanoparticles

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As applications of nanoscience and nanotechnology in a wide-range of commercial uses continue to expand, there is growing interest in understanding the environmental and health implications of nanomaterials. Inhalation of manufactured nanomaterials may be one potential route for nanoparticle exposure to humans. The implications of exposure to these airborne nanoparticles need to be determined through exposure studies of well-characterized nanoparticles. By evaluating which materials are more likely to cause deleterious health effects before large amounts are introduced in the environment, the risk and resources that would be devoted toward trying to eliminate and replace the materials may be avoided. In this research, we have fully integrated studies of the physical and chemical properties of commercially manufactured nanoparticles with inhalation toxicological studies of these same nanoparticles to determine those properties that most significantly affect toxicity. Using murine models for inflammation, inhalation exposures of CuO/Cu, Fe_2O_3/Fe, and TiO_2 nanoparticles were investigated to determine how size and composition affects inflammatory response.

EPA Grant Number: R831717

Hysteretic Accumulation and Release of Nanomaterials in the Vadose Zone

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Objectives: Manufactured nanomaterials are increasingly being considered for use in a wide range of applications, and their use is projected to expand substantially during the next 10 years as costs decrease and new applications are discovered. At present, little is known about the fate, transport, or transformation of nanomaterials in the environment, or their inherent risks to human or environmental health. The objective of this project is to study the vadose zone accumulation and release of a wide range of manufactured nanomaterials, with emphasis on hysteretic interactions with air/water interfaces and specific mineral surfaces. Nanomaterials can enter the vadose zone through infiltration of atmospheric dispersions, or from groundwater contaminated by landfill leachate or other sources. Depending on the nature of the materials and interactions with critical interfaces, the vadose zone may either provide a sink for nanomaterials, preventing their spread throughout the environment, or become a long-term contaminant source.

Approach: This research project will be conducted through three primary tasks. Task 1 (batch adsorption/adhesion experiments) is designed to assess adsorption/adhesion affinities with critical liquid/solid and liquid/air interfaces. Task 2 (saturated deposition/dispersion transport experiments) is designed to evaluate dynamic interactions between nanomaterials and mineral surfaces. Task 3 (dynamic hysteretic unsaturated transport experiments) is designed to provide detailed information about the effects of wetting/drying history, infiltration, and unsaturated soil behavior on the accumulation and release of nanomaterials. These tasks use a range of experimental systems to study specific mechanisms influencing the dynamic accumulation and release of manufactured nanomaterials in the vadose zone, and make extensive use of inline detectors to simultaneously track concentration, particle size, and zeta potential distributions. A novel technique for measurement of air/water interfacial area throughout hysteretic wetting/drying cycles will provide fundamental experimental information about the role of wetting state history and air/water interfacial areas in the accumulation and release of nanomaterials. Nanomaterials selected for this work cover a wide range of structures, compositions, and physical and chemical properties, in addition to different potential applications. The solid media selected for this work will include fully characterized whole soils and aquifer materials, as well as critical mineral subsets of the whole materials. An unsaturated flow and transport modeling effort conducted as a part of Task 3 will integrate the results of experimental tasks.

Expected Results: This research project will provide significant benefits to society in terms of improved *a priori* assessment of manufactured nanomaterial mobility in the environment and associated risk. Outcomes of the work will provide indications about the classes of nanomaterials most likely to accumulate in the vadose zone, the roles of mineral surfaces, air/water interfacial areas, and wetting/drying history on accumulation. This work will provide essential new information necessary to assess the mobility of manufactured nanomaterials in the environment and the role of vadose zone interactions in decreasing or increasing ultimate risk to human or environmental health.

EPA Grant Number: R832529

Carbon-Based Nanomaterials

Role of Particle Agglomeration in Nanoparticle Toxicity

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Objective: The objective of this study is to determine the biological consequences of nanoparticle agglomeration. We hypothesize that there will be a difference in the toxicity of fresh (predominantly singlet) versus aged (predominantly agglomerated) carbon nanoparticles, and in testing this hypothesis we will: (1) measure the agglomeration rate of several types of carbon nanoparticles; (2) identify whether agglomeration is affected by differing exposure conditions, including humidity and particle charge; and (3) compare the toxicity of singlet versus agglomerated particles in mice exposed via the inhalation route. A number of investigators have clearly demonstrated in instillation studies that nanoparticle toxicity is governed, in part, by particle size. Our preliminary studies have demonstrated that freshly formed nanoparticles produce lung injury and inflammation in mice, and the extent of adverse effects is influenced by genetic host factors. We will expand upon these findings and identify whether realistic exposure conditions, which lead to carbon nanoparticle agglomeration, alter the pulmonary response in mice. Particle agglomeration of nanoparticles is known to be influenced by number concentration and other physical factors. Almost all particle agglomeration data have been derived, however, under static conditions, whereas occupational exposure to nanoparticles occurs under dynamic conditions. It is critical, therefore, that the influence of agglomeration on nanoparticle toxicity be examined under dynamic conditions.

Approach: To test the hypothesis that there is a difference in the toxicity of fresh (predominantly singlet) versus aged (predominantly agglomerated) nanoparticles, we first will establish the agglomeration of freshly generated carbon nanoparticles at various distances (i.e., aging times) downstream from particle generation in a dynamic exposure system. After careful initial characterization of singlet and agglomerated particles, inbred mice will be exposed to nanoparticles (generated in an arc furnace) at various stages of particle agglomeration, and the lungs will be examined for injury and inflammation. To ensure that pulmonary differences in response are due to particle agglomeration, groups of mice will be exposed to singlet or agglomerated particles at the same time, using the same operating conditions and control of humidity and particle charge. To determine whether initial findings for a single type of particle composition are applicable to other nanoparticles, we also will generate particles with different amounts of metal content, as found in carbon nanoparticles generated by metal catalysts.

Expected Results: As determined in preliminary studies, we expect that nanoparticle toxicity will be influenced by a variety of exposure conditions, including particle size, number, agglomeration state, charge, and composition. By careful characterization of particle agglomeration in a dynamic system, our inhalation toxicity data should provide key information regarding the toxicity of emerging nanoparticle technologies. The data obtained in the proposed animal studies can readily be used for extrapolation to occupational and ambient settings. In summary, the results from this project will address a number of the research needs identified in this solicitation, including toxicity and exposure assessment.

EPA Grant Number: R832528

Chemical and Biological Behavior of Carbon Nanotubes in Estuarine Sedimentary Systems

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Objectives: The general objectives of this research project are to: (1) determine factors controlling the fate of single-walled carbon nanotubes (SWNTs) and their synthetic byproducts in estuarine seawater, sediment, and sediment-ingesting organisms; (2) examine the impact of SWNTs and byproducts on the disposition of model organic contaminants in estuarine sediments; (3) determine whether the presence of SWNTs and byproducts in estuarine sediments affects the bioavailability of model organic contaminants to estuarine invertebrates; and (4) assess the toxicity of SWNTs and byproducts to suspension- and deposit-feeding estuarine invertebrate models in seawater suspension alone and/or in combination with estuarine sediments.

Approach: Our research plan will address these objectives through a series of experiments designed to provide a holistic picture of the behavior of SWNTs and their synthetic byproducts upon entry into the estuarine environment. These experiments will include tracing the fate and phase-association of ^{14}C-SWNTs and byproducts under simulated estuarine conditions and through ingestion by deposit-feeding organisms; batch sorption studies to examine the affinity of SWNTs for model hydrophobic organic contaminants (HOC) in the estuarine environment; laboratory-scale bioaccumulation experiments designed to test modulation of HOC bioavailability by co-occurring SWNTs in estuarine sediments; and dose-response experiments designed to test the potential for SWNTs and byproducts to directly cause adverse effects on a sensitive estuarine infaunal invertebrate (the harpacticoid copepod *Amphisascus tenuiremus*).

Expected Results: This project will, for the first time, address the physical, chemical, and biological behavior of novel and emerging carbon nanotube materials under environmental conditions typical of estuaries. In total, we will address not only the potential for SWNTs to be transported, accumulate, and cause direct deleterious effects within estuarine environments, but also the potential for linked effects on the biological and chemical behaviors of known priority pollutants common in estuarine sediments. This combined approach represents a novel way of addressing the environmental impact of an emerging synthetic nanomaterial, and thus will provide the U.S. Environmental Protection Agency and the scientific community with an entirely new and highly relevant dataset for risk assessment of SWNT-derived contaminant discharge. Further, the work will generate new scientific knowledge related to the behavior of these highly novel nanomaterials under conditions not normally tested in the course of nanoscience research (e.g., nonmammalian biological systems, highly saline aqueous solutions, and complex sediment media). This knowledge may become useful in designing new nanoscale technologies in, for example, environmental engineering or “green” manufacturing techniques.

EPA Grant Number: R831716

Fate and Transformation of C_{60} Nanoparticles in Water Treatment Processes

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The environmental impact of carbon fullerenes is of great concern due to projections for bulk production in the near future and the recent discovery that they form nanoscale water-stable aggregates upon release to water. Understanding the fate and the transformations of carbon fullerenes during water treatment, currently our first line of defense against ingestion pathways, is of particular importance. Human exposure to these materials via water ingestion will be strongly influenced by the behavior of these aggregates in potable water treatment systems.

Objective: The objective of this research project is to examine the response of water-stable fullerene aggregates to processes that are used in potable water treatment, using C_{60} and its stable aggregate, nano-C_{60}, as a model compound. More specifically, this project will test the following hypotheses:

- Nano-C_{60} with an electron-rich surface will undergo chemical transformation through addition of oxygen or chlorine atom and/or charge destabilization when subjected to oxidation by commonly used oxidants and disinfectants such as ozone, UV light, free chlorine, and monochloramine.
- A unique, weakly negatively charged surface of nano-C_{60} will lead to unique electrostatic and hydrophobic interactions with metal hydroxide-soluble complexes and precipitates, with polymeric membrane surfaces, and with hydrophobic surfaces of activated carbon.
- The size characteristics of nano-C_{60} will lead to unique filtration characteristics when filtered through nanoporous membranes and unique adsorption kinetics/equilibrium characteristics when adsorbed by activated carbons with varying pore-size distributions.

Expected Results: The outcome of this research project will provide basic, fundamental, yet practical knowledge in chemical and physical behavior of this nanomaterial during commonly practiced engineering processes. New information, such as colloidal stability, chemical reaction kinetics, reaction product identity, transport behavior, and adsorptive characteristics, will advance scientific knowledge in use, disposal, and treatment of this growing class of materials and will trigger additional research on water treatment technologies and facilitate appropriate toxicological studies.

EPA Grant Number: R832526

Cross-Media Environmental Transport, Transformation, and Fate of Manufactured Carbonaceous Nanomaterials

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Despite the rapid growth in nanotechnology, very little is known about the unintended health or environmental effects of manufactured nanomaterials. The results of several recent studies suggest that manufactured nanomaterials may be toxic. Because experience with naturally occurring nanoscale particles present in air has shown that they are hazardous to human health and that they can easily travel global-scale distances in the atmosphere, such scenarios involving engineered nanoparticles must be explored. This research project seeks to examine carbonaceous nanomaterial fate and transport in the environment. In particular, we are interested in how these particles behave when transferred from water to air or vice versa. This project focuses on the characterization of aqueous aggregates of C_{60} fullerene.

The discovery that negatively charged aggregates of C_{60} are stable in aqueous environments has elicited concerns regarding the potential environmental and health effects of these aggregates. Although many previous studies have used aggregates synthesized using intermediate organic solvents, this project employed an aggregate production method believed to emulate more closely the fate of fullerene upon accidental release—extended mixing in water. The aggregates formed by this method are heterogeneous in size (20 nm and larger) and shape (angular to round), but are crystalline in structure, exhibiting a face-centered cubic (FCC) habit as determined by electron diffraction. In addition, particle shape and surface charge changed when C_{60} was mixed in the presence of electrolytes (NaCl, $CaCl_2$) or sodium citrate at concentrations from 1 to 100 mM. These changes in solution composition affect aggregate formation and stability and suggest that C_{60} fate and transport will be a function of the composition of the solution.

EPA Grant Number: R832534

Transport and Retention of Nanoscale Fullerene Aggregates in Water-Saturated Soils

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The goal of this research project is to advance our understanding of nanoscale fullerene (n-C_{60}) aggregate transport and retention in porous media through a combination of experimental and mathematical modeling studies. The specific objectives of this research are to: (1) quantify the fate and transport of crystalline n-C_{60} aggregates in water-saturated soils as a function of soil properties and systems parameters; (2) investigate the effects of C_{60} fullerene on soil water retention, water flow, and transport in unsaturated soils; and (3) develop and evaluate numerical models to describe carbon nanomaterial transport, retention, and release in subsurface systems.

Stable aqueous suspensions of n-C_{60} aggregates were prepared by dissolving fullerene in tetrahydrofuran (THF), which was mixed with an equal volume of water, evaporated at 75^{o}C, and sparged with N_2 gas. The resulting suspension contained approximately 3.0 mg/L of n-C_{60} aggregates with an average diameter of 95 nm, as determined by dynamic light scattering (DLS). In the first set of experiments, a pulse of suspended n-C_{60} solution was introduced into water-saturated columns packed with either 40-50 mesh glass beads or Ottawa sand at a Darcy velocity of 2.8 m/d. Effluent samples were collected continuously and analyzed by ultraviolet (UV) spectrometry to determine the C_{60} concentration, and by DLS to monitor changes in C_{60} aggregate size. Following each experiment, the column was sectioned into 1-cm increments, extracted in water with sonication, and analyzed by UV spectrometry to determine n-C_{60} retention profiles. In the presence of 1.0 mM $CaCl_2$, n-C_{60} effluent concentrations gradually increased to a maximum value and then decreased sharply upon re-introduction of the n-C_{60}-free solution. Retention of n-C_{60} in the glass bead columns ranged from 8 to 49 percent of the introduced mass, while up to 77 percent of the injected mass was retained in Ottawa sand columns. The observed retention capacities were consistent with the delayed breakthrough of n-C_{60} observed in the Ottawa sand columns and were corroborated by batch retention measurements. In the absence of background electrolyte, effluent n-C_{60} concentrations coincided with those of a nonreactive tracer (Br^-), demonstrating the important role of electrostatic interactions in n-C_{60} transport and retention. A second set of n-C_{60} transport experiments was conducted at several pore-water velocities in columns packed with various size fractions of Ottawa sand. Decreasing flow rate and smaller grain size resulted in greater n-C_{60} retention, with nearly complete retention observed with 80-100 and 100-140 mesh Ottawa sand.

The n-C_{60} effluent concentration and retention data were simulated using a mathematical model that incorporated nonequilibrium attachment kinetics and a limiting retention capacity term. The numerical model successfully captured the characteristics of both the effluent concentration and particle retention profiles. Experimental and simulation results suggest that n-C_{60} aggregate attachment is strongly dependent on porous media surface area and flow rate. Simulated attachment capacity increased with increased specific surface area, and for a given sand size fraction, simulated n-C_{60} attachment rates were greater at higher flow rates. Extended Derjaguin-Landau-Verwey-Overbeek (DLVO) theory, which included van der Waals, electrostatic repulsion, and hydrophobic interaction forces, was used to evaluate potential mechanisms governing n-C_{60} attachment. This analysis suggests that an energy barrier of about 27 kT exists between n-C_{60} aggregates and Ottawa sand surfaces, with a secondary minimum attraction region of 0.3 kT. Attachment rate coefficients derived from secondary energy minimum theory were found to be in close agreement with those fit using the numerical model. Additional studies are being conducted to further elucidate the mechanisms responsible for n-C_{60} transport and retention as a function of ionic strength, grain size, flow rate, and the presence of stabilizing agents.

EPA Grant Number: R832535

Repercussion of Carbon-Based Manufactured Nanoparticles on Microbial Processes in Environmental Systems

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The use of nanotechnology has tremendous potential for economic growth and is a key feature of sustainable development. Despite the impending increase in industrial production and the certain releases of Carbon-Based Manufactured Nanoparticles (CMNP) to the environment, almost nothing is known about their environmental impact. To engage in a publicly transparent evaluation of risks and benefits and to develop public policy and technology to manage potential risks, fundamental scientific environmental research must be completed. The goal of this research project is to provide fundamental information about the impact of CMNP on water, soil, and subsurface ecosystems.

Objective 1: We propose that there will be a shift in the structure of soil microbial populations in systems exposed to CMNP because the nanomaterial will exert pressure on the microbial population.

Approach: The intrinsic features describing activity will be estimated in four ways. We will: (1) draw information from the ratio of key fatty acids taken from the phospholipid fatty acids (PLFA) fraction and relate it to a background status of the soil microbial populations; (2) use genetic approaches (e.g., density gradient gel electrophoresis [DGGE] with both bacterial and fungal primers); (3) use enzyme assays for dehydrogenase, urease, and cellubiase; and (4) use respiration and trapping of CO_2 to estimate aerobic activity in the presence of the CMNP.

Objective 2: The long-term fate of CMNP in the environment and their entrance into soil and aquatic biogeochemical cycles will mostly be a function of the activity of the specific oxygenase, ligninase, laccase, and fenton systems resident in microbial populations.

Approach: Using ^{13}C-fullerenes in soil microcosm studies outlined in Hypothesis 1, we will track CMNP carbon to determine how the soil microbial biomass responds to CMNP. We also will assess the degree to which CMNP carbon is assimilated into microbial biomass, or is converted to a form bound with soil carbon. Additionally, we will inoculate various litter forms (wood and leaves) spiked with ^{13}C-labeled fullerene with aggressive decay fungi where our goal is to assess the degree to which CMNP carbon is assimilated into fungal biomass or converted to functionalized forms (free and bound).

Objective 3: Water-borne CMNP represent an, as yet, unassessed toxicological risk to aquatic organisms because of their capacity to physically interact with cell membranes and possibly causing harm to the cells.

Approach: We will use a lux-gfp-based assay to estimate the impact of the CMNP on the processes of respiration and growth, allowing us to arrive at the first CMNP structure-to-microbial function model. This objective will involve monitoring bacterial bioluminescence to evaluate the impact of CMNP (amount or structure) on bacterial response in aqueous systems.

Expected Results: The expected results of this research are very substantial. The knowledge gained from our research will be used by governments and industry for developing public policy and technology for the management of any environmental risks from CMNP. The research can be integrated with educational programs and used to disseminate knowledge about the behavior of nanomaterials.

EPA Grant Number: R831720

Size Distribution and Characteristics of Aerosol Released From Unrefined Carbon Nanotube Material

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Carbon nanotubes (CNTs) are among the most dynamic and fast-growing nanomaterials due to their novel properties. The potential of human exposure to this new type of material in the workplace, as well as in the general environment, is rising, and its impact on human health is of great concern.

In this study, we have investigated the size distributions of airborne CNT particles that were laboratory-generated by using a vortex agitator and dispersed with a very low flow of HEPA-filtered air. The number-weighted particle size distributions were monitored by a 13-stage Electrical Low Pressure Impactor (ELPI) and a 6-stage Integrating Screen Diffusion Battery (ISDB). Several industrial-grade unrefined CNT samples (raw materials) of various types have been examined, including single-walled, double-walled, and multiwalled nanotubes. The CNT samples were collected onto the aluminum substrates placed on each stage of the ELPI. For ISDB sampling, the samples were collected on an array of stainless steel screens, as well as mica discs attached on the wall between the screens. The experimental data demonstrated that all types of CNT raw materials examined can be dispersed into the air to a significant extent. The sizes of particles generated were widely distributed across all 13 stages of the ELPI, including the filter stage ranging from 7 nm to 10 μm. The ISDB results showed that the particles released from CVD-SWCNT material (HP-grade, Helix, TX) have a solo peak under 10 nm, with a mode of 2.5 nm and GSD of 1.24 in number-weighted distributions. The experimental data also showed that the size distributions varied with the type of CNTs and with the methods by which they were manufactured. The image analysis results by Atomic Force Microscopy showed that the CNTs tend to agglomerate rather than exist as single particles, physically.

These results suggest that CNTs can possibly become airborne under certain agitation conditions during manufacturing and handling processes and can expose workers via inhalation and dermal absorption. As deposition efficiency and sites of inhaled particles within the respiratory system largely depend on particle size distribution, the deposition pattern of agglomerated CNT should be similar to those larger, equivalent-sized nonagglomerated particles. Nevertheless, entrained particles depositing on/in the deep lung surfaces of the bronchioles or alveoli will contact pulmonary surfactants in the surface hypophase and the agglomerated CNT are likely to (ultimately) be de-agglomerated. Therefore, to investigate human exposure to airborne CNTs, the full-size range of inhalable particles must be taken into account.

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Physical and Chemical Determinants of Carbon Nanotube Toxicity

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There is real opportunity to reduce carbon nanotube health risks by understanding toxicity mechanisms and modifying the specific material features that trigger those mechanisms. This research project considers the role of two characteristic nanotube features: catalytic impurities and hydrophobic surface area. Electron micrographs show that most nanotube catalyst particles are encapsulated by carbon shells, which has led to the widespread impression that the metal is fluid inaccessible and unavailable for known biomolecular toxicity pathways. This project describes quantitative assays for the bioavailability of CNT nickel, iron, and yttrium in model extracellular fluids and phagolysosomal simulants. Toxicologically significant amounts of nickel and iron are released from 12 commercial nanotubes, both as-produced and "purified," and this bioavailability depends on material stresses (sonication, oxidation), physiological fluid properties (pH, ligands), and sample age. We also present preliminary work on the selective removal of the bioavailable portion of the metal as a potential detoxification strategy. Finally, amino acid and vitamin profiling is used to probe the effect of hydrophobic surface area on cell culture media. We find that single-wall nanotubes inhibit HepG2 cell proliferation by an indirect mechanism involving dose-dependent media depletion by physical adsorption of small-molecule solutes, especially folate.

EPA Grant Number: R831719

Environmental Impacts of Nanomaterials on Organisms and Ecosystems: Toxicity and Transport of Carbon-Based Nanomaterials Across Lipid Membranes

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The dramatic increase in production rates of nanomaterials (NM) and the anticipated widespread use of engineered nanoparticles in commercial and industrial applications suggest that NM will inevitably enter the environment, including the biosphere. These expectations stem from the high potential of nanotechnology to substantially benefit human societies by creating new means of detecting pollutants, cleaning polluted waste streams, recovering materials before they become wastes, and expanding the currently available resources, to name a few. In this research, we hypothesize that NM could lead to environmental dysfunctions due to: (1) the potential toxicity of these materials and their derivatives; (2) the nanometer-size that makes manufactured nanomaterials prone to biouptake/bioaccumulation; and (3) the large surface area which might lead NM to act as carriers/delivers of pollutants adsorbed onto them. Our objectives are to: (1) assess the toxicity of nanomaterials on biota using short-term micro-biotests and investigate the impacts of NM on microbial-driven ecological functions; (2) determine the mobility of metal-based and carbonaceous NM in porous media as well as the toxicity of NM in soil leachates; and (3) determine possible mechanisms of toxicity of different types of nanomaterials.

Through laboratory studies, the potential toxicity of tested NM was assessed using: (1) the *Ceriodaphnia* dubia acute toxicity assay; (2) the *Selenastrum capricornutum* (or *P. subcapitata*) chronic toxicity test; and (3) MetPLATE™, an enzyme-based test specific to metal toxicity. The impacts of NM on selected ecosystem functions, such as the microbial degradation of organic matter, were assessed using sediment slurries, while ongoing studies using soil columns investigated the fate and transport of NM in porous media. In addition to the above experimental work, a model is being developed to investigate the interactions of NM with cellular membranes. Our modeling studies are performed using a coarse-grained molecular dynamics (CGMD) model (molecular dynamics simulations), which approximates small groups of atoms as a single united atom.

In this study, we model cell membranes as lipid bilayers, thereby neglecting other constituents of the membrane, such as membrane proteins. This model for cell membranes is consistent with the experimental indication that interaction of NM with membrane lipids plays a dominant role in mechanisms of cytotoxicity. Although our ongoing research deals with nanometallic particles (e.g., Ag, Cu, Co, Ni, and nano-metal oxides), quantum dots (e.g., CdSe, CdS), and carbonaceous NM (i.e., C_{60}, SWNT, MWNT), this presentation will be limited to C_{60} and model carbon nanotube data only.

Based on the above-mentioned toxicity tests, we first examined the toxicity of different solvents (e.g., THF, SDS, SDBS, PVP, Triton X-100, Triton X-15, Sodium cholate, Gum Arabic) that potentially could be used to obtain highly dispersed fullerene suspensions. Our results show that most solvents are very toxic, even at trace levels (e.g., 0.005% V/V). Therefore, the presence of trace levels of toxic solvents in samples used in organism-based toxicity tests easily could lead to erroneous results. Second, using aqueous C_{60} suspensions prepared by a procedure adapted from that of Degushi (by making sure that the residual THF level in control water samples, if any, produces no toxicity), both the invertebrate- and algal-based toxicity tests showed the negative impact of tested NM, with the algal-based test being more sensitive than the invertebrate-based test. The prepared aqueous C_{60} suspension also was used to spike both lake and wetland sediments. In this latter case, a clear negative impact on rates of acetate decomposition by microbial sedimentary processes was observed. Third, the computational investigation of molecular mechanisms of NM toxicity focusing on interactions of nanoparticles with cell membranes is being conducted, in parallel with the above-described laboratory experiments. For model carbon-based NM (C_{60} and carbon nanotubes), we observed an extremely

small barrier for the permeation of the NM into the hydrophobic interior of a lipid bilayer. Conversely, the calculated residence time of the NM within the bilayer interior is very large, which possibly could lead to destabilizing interactions between NM and the membrane. We have initiated theoretical studies to assess possible physical and chemical mechanisms of the membrane disruption by NM, and our preliminary results will be discussed.

EPA Grant Number: R832635

Structure–Function Relationships in Engineered Nanomaterial Toxicity

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Objective: As nanotechnology develops into a mature industry, the environmental and health effects of its core materials are of increasing importance. A significant challenge for this area of research is that for every class of engineered nanoparticle (nanotubes, metal nanocrystals), there are literally thousands of possible samples with various sizes, surfaces, and shapes. This huge parameter space cannot be narrowed by focusing only on commercial materials, as few systems are in commerce at this point. Indeed, most nanotechnology companies are optimizing and evaluating hundreds of material prototypes for possible commercial use. In such a climate, all stakeholders benefit from an understanding of how fundamental nanoparticle characteristics (e.g., surface chemistry, size, shape) control their biological effects.

This aim is the overarching objective of this research project, which stated another way, will provide the first structure–function relationships for nanoparticle toxicology. This information benefits industry in that it will suggest material modifications that may produce systems with minimal environmental and health impact. It also benefits regulators by not only indicating whether information on one nanoparticle type can be used to predict the properties of a related material, but also by setting a framework for evaluating newly developed nanoparticle variants. Finally, a correlation between biological effects and nanoparticle structure will enable the development of chemical methods to alter more toxic nanomaterial species into less toxic materials upon disposal.

To realize these structure–function relationships requires that we develop new analytical tools as well as evaluate material datasets with systematic changes in fundamental properties. Our specific objectives are to: (1) expand the characterization of nanoparticle structure in biological media; and (2) characterize the effects of nanoparticles on cell function. This data will be used to test the hypothesis that nanoparticle structure (e.g., size and shape) directly controls cytotoxicity. A secondary hypothesis is that of the four major materials parameters in engineered nanoparticles (size, shape, composition, and surface), surface will be the most important in governing cellular effects. These hypotheses will be tested in several major classes of nanoparticles.

Approach: This project exploits recent advances in nanochemistry, which allow for the production of highly size- and surface-controlled nanoparticles from a variety of materials. These model systems provide the systematic variations in nanoparticle "structure" required for structure–function relationships. Our model systems will include engineered carbon nanoparticles, both C_{60} and single-walled carbon nanotubes, up to eight distinct sizes of nanoscale iron oxides, and a wide variety of nanoscale titania with varying surface coatings. All of these materials have been reported to generate oxygen radicals under some circumstances; thus, we expect to correlate our "structures" with the acute cellular toxicity in three human cell lines. This overarching objective is strongly supported by ongoing efforts to expand the characterization of nanoparticle structure directly in biological media (objective #1). Additionally, structure–function trends are made much more general if they can be rationalized by some basic mechanism. Thus, objective #2 aims to both characterize nanoparticle–cell interactions as well as put forward a mechanism to explain any observed acute toxicity.

Expected Results: The introduction of a new class of materials into consumer products will require information about the potential behavior and risks these systems pose to the environment and people. Risk management will be improved with the information provided in this grant, particularly in that we will establish structure–function relationships for several major classes of nanomaterials.

EPA Grant Number: R832536

Interactions of Pure and Hybrid Polymer Nanofibers With Cells

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Nanostructured materials, such as natural polymers, are commonly used to build scaffolds that enable cell growth and proliferation while supporting cell differentiation. This research project addresses the need for assessing the cell-nanomaterial interactions. It is believed that the degree of cell attachment to the scaffold has a direct influence on cell motility, proliferation rate, and control of phenotype. In this study, the nature of osteoblast attachment to nanostructured fibers of pure cellulose acetate (CA) and cellulose acetate reinforced with hydroxyapatite nanoparticles (CA-HA) is being reported. The fibrous mats were prepared by means of electrospinning, a potent nanomanufacturing technique. Osteoblast cells (SaOS-2) were seeded on the electrospun mats at a density of about 68,000 cells/well. CA-HA composite scaffolds appeared to favor cell spreading, with hydroxyapatite nanoparticle aggregates enhancing cell attachment to the fibers by providing anchoring sites.

EPA Grant Number: R832537

Other Nanomaterials

Cellular Uptake and Toxicity of Dendritic Nanomaterials: An Integrated Physicochemical and Toxicogenomics Study

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Objective: Dendrimers are relatively monodisperse and highly branched nanoparticles that can be designed to chelate metal ions, encapsulate metal clusters, bind organic solutes or bioactive compounds, and become soluble in appropriate media or bind onto appropriate surfaces. Because of these unique properties, dendrimers are providing unprecedented opportunities to develop functional nanomaterials for a variety of applications, including chemical separations and catalysis, chemical sensing, medical imaging, DNA/drug delivery, and water purification. As the U.S. Environmental Protection Agency (EPA) begins its assessment of the impact of nanotechnology on human health and the environment, there is a critical need of data and quantitative tools for assessing the environmental fate and toxicity of nanomaterials, such as dendrimers. The overall objective of this research project is to advance our fundamental understanding of the relationships between the affinity of ethylene diamine (EDA) core poly(amidoamine) PAMAM dendrimers to cell membranes and their vascular and ingestion toxicity using: (1) n-octanol and solid-supported phosphatidyl-choline lipid bilayers as model cell membranes; and (2) endothelial and kidney cells as model human cells.

Approach: To achieve this overall objective, we propose to implement an integrated physical-chemical and toxicogenomics study that combines: (1) dendrimer synthesis and characterization; (2) measurements of the octanol–water and liposomes–water partition coefficients of EDA core PAMAM dendrimers at physiological pH; (3) AFM imaging of dendrimer interactions with liposomes at physiological pH; (4) molecular dynamics (MD) simulations to determine the physical-chemical properties (e.g., size, shape, internal structure, and extent of hydration, etc.) of EDA core PAMAM dendrimers in aqueous solutions at physiological pH; and (5) experimental characterization of the vascular and ingestion toxicity of dendrimers through *in vitro* measurements of cell viability and toxicogenomics studies of human endothelial and kidney cells exposed to aqueous solutions of dendrimers at physiological pH.

Expected Results: The successful completion of this project is expected to provide industry with critical data and predictive tools needed to assess the health and environmental impact of dendritic nanomaterials, such as EDA core PAMAM dendrimers.

EPA Grant Number: R832525

Assessment of Nanoparticle Measurement Instruments

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A typical industrial hygiene analysis of workplace dust exposure does not include instrumentation to detect particles in the nanometer size range. One of the goals of this research project is to compare a suite of aerosol measurement instruments for the purpose of demonstrating their differences and similarities to more effectively evaluate workplaces that may have a nanoparticle aerosol. The instruments analyzed include a scanning mobility particle sizer, portable condensation particle counter, surface area monitor, photometer, and optical particle counter. The measurements made by these instruments were compared to mass concentration measurements made by gravimetric analysis, and count concentration and size distribution made by transmission electron microscopy. All instruments are connected via ports attached to a 20 L sealed chamber acting as a plenum through which dilution air flowed at 25-L/min. Prior to this work, an assessment of various methods for aerosolizing nanoparticles from the bulk powder were compared. These methods included both dry powder dispersers and nebulization of a liquid suspension and involved powders consisting of titanium dioxide, iron oxide, silicon dioxide, and single-walled carbon nanotubes. Polystyrene latex spheres with diameters less than 100 nm also were tested as a control for particles with known geometry and size distribution. Multiple trials of each dust type were conducted, and t-tests were used to perform pair-wise comparisons of instrument output for instruments that were directly comparable. Conversions were made to some measurements to compare, for example, count measurements with surface area measurements. The results indicate a need to apply a shape factor to make direct correlations between instruments, especially when comparing between instruments with different units—count, surface area, or mass concentrations. This information will be useful for comparing results obtained by different instruments and for choosing an appropriate instrument for evaluation of nanoparticles in the workplace.

NIOSH Grant Number: R01 OH008806

Development of Nanosensors for the Detection of Paralytic Shellfish Toxins (PSTs)

Robert Gawley
University of Arkansas, Fayetteville, AR

This research project will focus on progress in the development of nanosensors for detection of paralytic shellfish toxins (PSTs), of which saxitoxin is the parent. Our recent efforts have focused on the following: (1) determining the PST profile in blue mussels from Puget Sound in the summer of 2006; (2) correlating the fluorescence response of our chemosensors to the blue mussel extracts in solution; and (3) incorporating our chemosensors into a self-assembled monolayer for incorporation into a sensing device.

EPA Grant Number: GR832382

Transformations of Biologically Conjugated CdSe Quantum Dots Released Into Water and Biofilms

Patricia Holden[1] and Jay L. Nadeau[2]
[1]University of California–Santa Barbara, Santa Barbara, CA;
[2]McGill University, Montreal, Quebec, Canada

Objective: Semiconductor nanocrystals (quantum dots) differ in important ways from bulk semiconductor materials. Their increased band gap means that they function as strong oxidizing and/or reducing agents, and their small size allows them to pass into living cells. Conjugation of biomolecules to the crystal surface can alter any or all of these properties. In preliminary experiments, we have observed that nucleobase-conjugated CdSe quantum dots (QDs) were actively taken up by soil and aquatic bacteria (for example, *Bacillus subtilis* and *Escherichia coli*). Effects on microbial viability attributed to the presence of the QDs included slower doubling times, heavy metal sequestration, and "blebbing" of metals into the environment. We propose to quantify these effects using a variety of biologically conjugated QDs and an assortment of microbial species, monitoring the process of QD uptake and breakdown and characterizing the breakdown products that result from bacterial metabolism of these particles. Possible hazards to microbial populations with extrapolation to humans through contamination of soil and water with QD breakdown products will be analyzed and quantified.

Approach: Bare, core-shell, and biologically conjugated QDs will be studied. Abiotic breakdown kinetics and products in aqueous environments will be determined by inductively coupled plasma (ICP) spectrometry for QDs as a function of exposure to light, pH, and oxidizing or reducing conditions. In preliminary experiments, biologically conjugated QDs are easily taken up by *B. subtilis*, but the process is light and pH-dependent. Some breakdown occurs inside and outside of cells. Working with *Pseudomonas aeruginosa* and *Staphylococcus aureus* to represent Cd-sensitive and Cd-resistant strains, we will quantify population growth and fluorescence for pure liquid cultures previously exposed to QDs. Conventional methods (shake flask, viable and direct counting over time) will be used to assess the effects of labeling on bacterial growth rates under high- and low-nutrient conditions. QD fluorescence will be monitored throughout, and final results will be adjusted for the dilution effect of growing populations. Concentrations of Cd and Se will be assessed inside and outside cells, and membrane associations of whole QDs and breakdown products will be quantified. The relationship of QD release and breakdown to cell viability will be assessed. DNA damage in bacteria will be assessed by quantifying 8-oxoguanine, a product of oxidative DNA damage, by microscopy and a commercially available fluorescent label. These experiments will provide basic insight into cellular interactions with QDs. The potential for single-base pair damage from whole QDs and breakdown products will be assessed using time-correlated, single-photon counting techniques. Because most bacteria exist as biofilms in nature, we will culture mono- and dual-species bacterial biofilms under continuous flow conditions in a commercially available flow cell. Using digital photomicroscopy and computerized image analysis, we will assess the effects of QD labeling on biofilm growth. Unsaturated biofilms also will be cultured on membranes to assess the effects of QD labeling on development under soil-like conditions, and as a function of nutrient and water availability. Cryo-environmental scanning electron microscopy (ESEM), coupled with energy dispersive spectrometry (EDS), will be used to visualize ultrastructural QD associations. Biofilms cultured in the absence of QDs will be exposed under a range of experimental conditions and assessed over time for viability and QD content. For all biofilm experiments, QD effects on exopolymeric substances (EPS) can be quantified by GC-MS of derivatized glycosyl residues, and DNA and protein content determined by standard fluorometric and colorimetric methods, respectively. Finally, column studies, using packed porous media under saturated and unsaturated conditions, will be conducted to assess QD and Cd mobility as a function of bacterial colonization. Because EPS is expected to chelate Cd, we will quantify whole QDs, Cd, Se, and biopolymers in breakthrough experiments, followed by sacrificial characterization of residual analytes.

Expected Results: For a range of conditions and for a variety of environmental factors, we will discover the fates and interactions of bare, core-shell, and conjugated CdSe QDs with bacteria. We will discover QD effects on bacteria and DNA and differentiate effects of QDs from the effects of the independent metal species. Both well-mixed liquid culture and biofilm modes of bacterial cultivation will be used, reflecting the full range of planktonic to attached modes of growth in nature. Experiments also will be performed with porous media

columns to quantify how bacterial colonization affects the transport and fate of QDs. This research project will provide a comprehensive investigation into bacterial QD interactions, which is imperative to understand the impact and fates of these nanoparticles in the environment. This work is necessary for comprehending the environmental fates and impacts of QDs, which are increasingly widespread devices in nanotechnology.

EPA Grant Number: R831712

Nanotechnology: A Novel Approach To Prevent Biocide Leaching

Patricia Heiden[1], Benjamin Dawson-Andoh[2], and Laurent Matuana[1]
[1]Michigan Technological University, Houghton, MI; [2]West Virginia University, Morgantown, WV

Objective: The primary objective of this research project is to develop a practical and effective approach to prepare biocide-loaded nanoparticles (organic and copper-based biocides) that can be efficiently introduced into wood to reduce or eliminate biocide leach into sensitive environments. Preventing biocide loss to leach also is expected to increase the useful lifetime of wood products while using less biocide. To accomplish this objective, the nanoparticle must be constructed not only to serve as a protective reservoir for the biocide that prevents its loss by leach or by degradation, but also to release biocide into the wood in a controlled manner, at a rate that maintains the minimal amount of biocide required within the wood for wood preservation.

Approach: A new nanoparticle preparation method is being developed to prepare hydrophobic nanoparticles that serve as a biocide reservoir and will moderate the biocide release rate. The nanoparticles will be stabilized in water so that they may be delivered into wood using a conventional, modified, full pressure-treatment method. American Society for Testing and Materials (ASTM) and American Wood Preservers' Association (AWPA)-approved methods, respectively, will be used to determine the biological efficacy of treated sapwood of pine and birch against the brown rot fungus, *Gloeophyllum trabeum,* and the white rot fungus, *Trametes versicolor,* and the leach rates of biocide from the nanoparticle-treated wood. Wood controls will be prepared by treatment with the same amount of biocide introduced by conventional solution or emulsion methods and evaluated in the same tests in side-by-side studies. All results will be compared and assessed for statistically significant differences.

Expected Results: This project will demonstrate the environmental benefits of introducing biocide into wood using hydrophobic nanoparticles as a delivery vehicle and controlled release device for organic and inorganic biocides. The primary benefits expected from use of nanoparticles as controlled release devices for biocide in wood are an increased service life of wood and a reduction of biocide loss to leach, which is expected to allow wood to be effectively protected with lesser amounts of biocide than is used now. These benefits are expected to be realized by using a new and more efficient nanoparticle preparation to give a slow biocide release rate coupled with good nanoparticle stability in aqueous suspensions. These features will allow the nanoparticles to be delivered efficiently into wood, but once in wood, to maintain a slow release rate. Successful completion of this project will benefit all ecosystems containing preserved wood. Even greater benefits are expected for wetlands and other moist ecosystems through reduction of biocide contamination, and in forest ecosystems harvested for wood by extending the service life of preserved wood and wood products.

EPA Grant Number: GR832371

Evaluating the Impacts of Nanomanufacturing Via Thermodynamic and Life Cycle Analysis

Bhavik R. Bakshi and L. James Lee
Ohio State University, Columbus, OH

Objective: This research project will develop original life cycle inventory data for the manufacture of polymer nanocomposites; test two new hypotheses for thermodynamics-based life cycle assessment (LCA) and impact assessment with limited information; and develop a tool for exploring economic and environmental aspects of alternate manufacturing combinations for selected nanoproducts and conventional processes. The following hypotheses will be tested: (1) among alternatives for making similar products, the one with a higher life cycle thermodynamic efficiency has a smaller life cycle impact; and (2) emissions with a smaller life cycle thermodynamic efficiency have a larger ecotoxicological impact. The second law of thermodynamics and hierarchical systems theory support these hypotheses. However, validating them has been challenging.

Approach: Through collaboration with leading academic groups, industry, and a national laboratory, life cycle inventory data and modules will be developed for the synthesis and use of nanoclays and carbon nanofibers. These modules will be combined with life cycle information at different spatial scales, ranging from equipment to ecosystems, and used to perform multiscale or hybrid LCA of several potential products. Different scenarios for the manufacture, use, end of life, emissions, and exposure of typical consumable and durable products, such as automotive body panels and food wrapping film, will be analyzed, along with estimates of uncertainty. Thermodynamic LCA will treat industrial and ecological systems as networks of energy flow and combine the features of systems ecology, LCA, and systems engineering. The proposed hypotheses will be tested in a statistical sound manner via several case studies.

Expected Results: LCA of nanotechnology is essential for guiding and managing risk in research, development, and commercialization while preventing irrational optimism or unfounded fear of this emerging field. However, it presents formidable obstacles because data and knowledge about resource consumption, emissions, and their impact are either unknown or not readily available. This project will lay the foundation for LCA of polymer nanocomposites and other emerging technologies. Validation of the first hypothesis will provide useful insight about nano versus traditional technologies, while the second hypothesis will provide a proxy for the ecotoxicological impact of the emissions. These hypotheses will be useful for nano and other emerging technologies before detailed emissions data and ecotoxicological studies are available. As more information about manufacturing, emissions, and their impact becomes available, it will be incorporated in the proposed studies and tool.

EPA Grant Number: R832532

Appendices

Interagency Workshop on the Environmental Implications of Nanotechnology

Hotel Monaco
700 F Street, NW
Washington, DC

September 5–7, 2007

AGENDA

DAY 1, Wednesday, September 5, 2007

8:00 – 8:45 a.m. **Registration**

8:45 – 9:00 a.m. **Welcome**
Gary Foley, Director, National Center for Environmental Research, Office of Research and Development (ORD), U.S. Environmental Protection Agency (EPA)

9:00 – 9:20 a.m. **Nanotechnology Environmental and Health Implications (NEHI), National Nanotechnology Initiative (NNI) Research Needs**
Celia Merzbacher, Assistant Director for Technology Research and Development, Office of Science and Technology Policy, Executive Director, President's Council of Advisors on Science and Technology

9:20 – 9:40 a.m. **Department of Energy (DOE) Research User Facilities**
Altaf H. Carim, Office of Basic Energy Sciences, DOE

9:40 – 10:00 a.m. **National Science Foundation (NSF)**
Cynthia J. Ekstein, Chemical, Bioengineering, Environmental, and Transport Systems Division, NSF

10:00 – 10:20 a.m. **National Institute for Occupational Safety and Health (NIOSH)**
Vladimir Murashov, Office of the Director, NIOSH

10:20 – 10:50 a.m. **National Institute of Environmental Health Sciences (NIEHS)**
Nigel Walker, National Toxicology Program (NTP), NIEHS

10:50 – 11:20 a.m. ***BREAK***

11:20 – 11:40 a.m. **Office of Research and Development Introduction**
George M. Gray, Assistant Administrator, ORD, EPA

11:40 – 11:50 a.m. **Science To Achieve Results (STAR) Nanotechnology Program**
Chris Saint, Division Director, ORD, EPA

DAY 1, Wednesday, September 5, 2007 (continued)

11:50 – 1:00 p.m.	***LUNCH***
	Metals, Metal Oxides
1:00 – 1:20 p.m.	**Fate, Transformation, and Toxicity of Manufactured Nanomaterials in Drinking Water** *Paul Westerhoff, Arizona State University*
1:20 – 1:40 p.m.	**Pulmonary and Systemic Inhalation Toxicity of Multiwalled Carbon Nanotubes** *Jacob McDonald, Lovelace Respiratory Research Institute*
1:40 – 2:00 p.m.	**Pharmacokinetics and Biodistribution of Quantum Dot Nanoparticles in Isolated Perfused Skin** *Nancy Monteiro-Riviere, North Carolina State University*
2:00 – 2:20 p.m.	**Metal Nanoparticle Tissue Distribution Following *In Vivo* Exposures** *Alison Elder, University of Rochester*
2:20 – 2:50 p.m.	***BREAK***
	Metals, Metal Oxides (continued)
2:50 – 3:10 p.m.	**The Bioavailability, Toxicity, and Trophic Transfer of Manufactured ZnO Nanoparticles: A View From the Bottom** *Jason Unrine, University of Georgia*
3:10 – 3:30 p.m.	**Uptake and Toxicity of Metallic Nanoparticles in Freshwater Fish** *David Barber, University of Florida*
3:30 – 3:50 p.m.	**Acute and Developmental Toxicity of Metal Oxide Nanoparticles to Fish and Frogs** *George Cobb, Texas Tech University*
3:50 – 4:10 p.m.	**Mechanistic Dosimetry Models of Nanomaterial Deposition in the Respiratory Tract** *Bahman Asgharian, CIIT Centers for Health Research*
4:10 – 4:30 p.m.	**Synthesis and Application of a New Class of Stabilized Nanoscale Iron Particles for Rapid Destruction of Chlorinated Hydrocarbons in Soil and Groundwater** *Dongye Zhao, Auburn University*
4:30 p.m.	***ADJOURN – DAY 1***

DAY 2, Thursday, September 6, 2007

8:30 – 8:40 a.m.	**Welcome**
	Metals, Metal Oxides (continued)
8:40 – 9:00 a.m.	**Nanostructured Materials for Environmental Decontamination of Chlorinated Compounds** *Vijay John, Tulane University*
9:00 – 9:20 a.m.	**Responses of Lung Cells to Metals in Manufactured Nanoparticles** *John Veranth, University of Utah*
9:20 – 9:40 a.m.	**A Toxicogenomics Approach for Assessing the Safety of Single-Walled Carbon Nanotubes in Human Skin and Lung Cells** *Mary Jane Cunningham, Houston Advanced Research Center*
9:40 – 10:00 a.m.	**Microbial Impacts of Engineered Nanoparticles** *Delina Lyon, Rice University*
10:00 – 10:20 a.m.	**An Integrated Approach Toward Understanding the Inflammatory Response of Mice to Commercially Manufactured CuO/Cu, Fe_2O_3/Fe, and TiO_2 Nanoparticles** *Vicki Grassian, The University of Iowa*
10:20 – 10:50 a.m.	***BREAK***
10:50 – 11:10 a.m.	**Hysteretic Accumulation and Release of Nanomaterials in the Vadose Zone** *Tohren Kibbey, University of Oklahoma*
	Carbon-Based Nanomaterials
11:10 – 11:30 a.m.	**Role of Particle Agglomeration in Nanoparticle Toxicity** *Terry Gordon, New York University School of Medicine*
11:30 – 11:50 a.m.	**Chemical and Biological Behavior of Carbon Nanotubes in Estuarine Sedimentary Systems** *Lee Ferguson, University of South Carolina*
11:50 – 12:10 p.m.	**Fate and Transformation of C_{60} Nanoparticles in Water Treatment Processes** *Jaehong Kim, Georgia Institute of Technology*
12:10 – 1:20 p.m.	***LUNCH***

DAY 2, Thursday, September 6, 2007 (continued)

Carbon-Based Nanomaterials (continued)

1:20 – 1:40 p.m.	**Cross-Media Environmental Transport, Transformation, and Fate of Manufactured Carbonaceous Nanomaterials** *Peter Vikesland, Virginia Tech*
1:40 – 2:00 p.m.	**Transport and Retention of Nanoscale Fullerene Aggregates in Water-Saturated Soils** *Kurt Pennell, Georgia Institute of Technology*
2:00 – 2:20 p.m.	**Repercussion of Carbon-Based Manufactured Nanoparticles on Microbial Processes in Environmental Systems** *Ronald Turco, Purdue University*
2:20 – 2:50 p.m.	***BREAK***

Carbon-Based Nanomaterials (continued)

2:50 – 3:10 p.m.	**Size Distribution and Characteristics of Aerosol Released From Unrefined Carbon Nanotube Material** *Judy Xiong, New York University School of Medicine*
3:10 – 3:30 p.m.	**Physical and Chemical Determinants of Carbon Nanotube Toxicity** *Robert Hurt, Brown University*
3:30 – 3:50 p.m.	**Environmental Impacts of Nanomaterials on Organisms and Ecosystems: Toxicity and Transport of Carbon-Based Nanomaterials Across Lipid Membranes** *Dmitry Kopelevich, University of Florida*
3:50 – 4:10 p.m.	**Structure-Function Relationships in Engineered Nanomaterial Toxicity** *Vicki Colvin, Rice University*
4:10 – 4:30 p.m.	**Interactions of Pure and Hybrid Polymer Nanofibers With Cells** *Perena Gouma, State University of New York–Stony Brook*

Other Nanomaterials

4:30 – 4:50 p.m.	**Cellular Uptake and Toxicity of Dendritic Nanomaterials: An Integrated Physicochemical and Toxicogenomics Study** *Mamadou Diallo, California Institute of Technology*
4:50 p.m.	***ADJOURN – DAY 2***

DAY 3, Friday, September 7, 2007

8:30 – 8:40 a.m.	**Welcome**
	Other Nanomaterials (continued)
8:40 – 9:00 a.m.	**Assessment of Nanoparticle Measurement Instruments** *Patrick O'Shaughnessy, The University of Iowa*
9:00 – 9:20 a.m.	**Development of Nanosensors for the Detection of Paralytic Shellfish Toxins (PSTs)** *Robert Gawley, University of Arkansas*
9:20 – 9:40 a.m.	**Transformations of Biologically Conjugated CdSe Quantum Dots Released Into Water and Biofilms** *Patricia Holden, University of California–Santa Barbara*
9:40 – 10:00 a.m.	**Nanotechnology: A Novel Approach To Prevent Biocide Leaching** *Patricia Heiden, Michigan Technological University*
10:00 – 10:20 a.m.	**Evaluating the Impacts of Nanomanufacturing Via Thermodynamic and Life Cycle Analysis** *Bhavik Bakshi, The Ohio State University*
10:20 a.m.	***MEETING ADJOURNMENT***

Interagency Workshop on Environmental Implications of Nanotechnology
September 5–7, 2007

Hotel Monaco
700 F Street, NW
Washington, DC

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How the National Nanotechnology Initiative is Addressing Environmental, Health & Safety Research Needs

Celia Merzbacher, Ph.D.
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Interagency Workshop on Environmental Implications of Nanotechnology
*5 September 2007 * Washington DC*

1

Budget for Environmental, Health, and Safety R&D, 2006–2008 (dollars in millions)

	2006 Actual	2007 Estimate	2008 Request
NSF	21.0	25.7	28.8
DOD	1.0	1.0	1.0
DOE	0.5	0.0	3.0
DHHS (NIH)	5.2	5.4	5.7
DOC (NIST)	2.4	2.8	5.8
NASA	0.0	0.0	0.0
EPA	3.7	7.9	9.6
USDA (CSREES)	0.1	0.1	0.1
DHHS (NIOSH)	3.8	4.9	4.6
USDA (FS)	0.0	0.0	0.0
DHS	0.0	0.0	0.0
DOJ	0.0	0.0	0.0
DOT (FHWA)	0.0	0.0	0.0
TOTAL	**37.7**	**47.8**	**58.6**

2

Planned 2008 Agency Investments by Program Component Area (dollars in millions)

	Fundamental Phenomena & Processes	Nanomaterials	Nanoscale Devices & Systems	Instr. Research, Metrology, & Standards	Nano-manufacturing	Major Research Facilities & Instr. Acquisition	Societal Dimensions	NNI Total
NSF	142.7	60.2	51.1	14.5	26.9	31.6	62.9	389.9
DOD	179.1	91.7	70.6	8.3	1.0	23.0	1.0	374.7
DOE	85.4	99.8	13.5	26.7	2.0	100.6	3.5	331.5
DHHS (NIH)	53.3	16.5	114.9	6.7	1.7	0.1	9.7	202.9
DOC (NIST)	27.1	8.0	13.5	26.4	11.1	4.5	6.0	96.6
NASA	1.0	12.0	10.0	0.0	1.0	0.0	0.0	24.0
EPA	0.2	0.2	0.2	0.0	0.0	0.0	9.6	10.2
USDA (CSREES)	0.4	0.8	1.5	0.0	0.1	0.0	0.2	3.0
DHHS (NIOSH)	0.0	0.0	0.0	0.0	0.0	0.0	4.6	4.6
USDA (FS)	1.7	1.5	1.0	0.2	0.2	0.0	0.0	4.6
DHS	0.0	0.0	1.0	0.0	0.0	0.0	0.0	1.0
DOJ	0.0	0.0	0.1	0.8	0.0	0.0	0.0	0.9
DOT (FHWA)	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.9
TOTAL*	491.8	290.7	277.4	83.6	44.0	159.8	97.5	1444.8

3

NEHI Working Group

- Subgroup of the NSTC Nanoscale Science, Engineering, and Technology Subcommittee
- Established in 2003
- Co-chaired by FDA & EPA/ORD
- Members include research and regulatory agencies
- Provides for information exchange
- Aims to identify and address EHS research needed to support regulatory decision making

4

EHS Research Categories

- Instrumentation, metrology & analytical methods
- Nanomaterials and human health
- Nanomaterials and the environment
- Health and environmental exposure assessment
- Risk management methods

5

Principles for prioritizing research

- Maximize value of information to be gained.
  - How much will uncertainty be reduced?
  - How broadly applicable will the information be?
  - What is the expected level of exposure?
- Seek to leverage investment against that of other stakeholders (e.g., industry, other countries)
- Be aware of the state of the art.

6

Next Steps

- Get public input on priorities
- Compare priorities with current research to identify any gaps and overlaps
- Develop research strategy to address unmet research needs.

See interim document at www.nano.gov

7

Some important points

- EHS research is a shared responsibility
- Research absolute effects, but also net risks
- Integrate risk research with development
- Research exposure as well as toxicity
- Develop standards
- Understand risk communication

8

Office of Science
U.S. Department of Energy

Department of Energy (DOE) User Facilities for Nanoscale Science: National Resources for Researchers

Dr. Altaf H. Carim
Scientific User Facilities Division
Office of Basic Energy Sciences

Interagency Workshop on the
Environmental Implications of Nanotechnology

Hotel Monaco, Washington, DC September 5, 2007

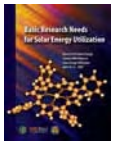
http://www.science.doe.gov/bes/

The National Nanotechnology Initiative, and DOE's role

- The National Nanotechnology Initiative (NNI) is an interagency program, started in 2001, that coordinates Federal nanoscale research and development activities and related efforts among 26 participating entities
- Planned federal NNI expenditures are over $1.4 billion in FY 2008

- The Department of Energy is one of the original participants in the NNI, and provides major funding for nanoscale science, engineering, and technology. The FY 2008 budget request includes over $285 million for nanotechnology in DOE's Office of Science, which supports both fundamental research and facilities.

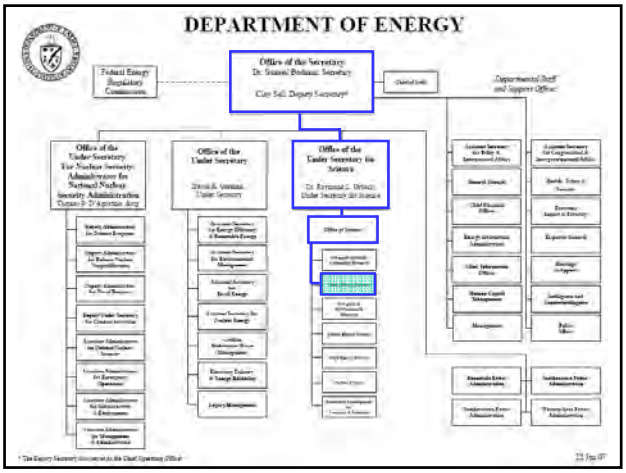
Research community needs drive DOE activities



- Energy & environmental grand challenge areas identified from the start of the National Nanotechnology Initiative in FY 2001
- DOE-SC-BES workshops cited nanoscience as a cross-cutting theme:
  - Basic Research Needs To Assure A Secure Energy Future (2002)
  - Basic Research Needs for the Hydrogen Economy (2003)
  - Basic Research Needs for Solar Energy Utilization (2005)
- Major NNI- and DOE-sponsored workshop in 2004 identified key research targets and foundational themes for energy-related nanoscience.

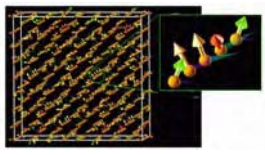
(All DOE-BES reports: see http://www.sc.doe.gov/bes/reports/list.html)

DEPARTMENT OF ENERGY



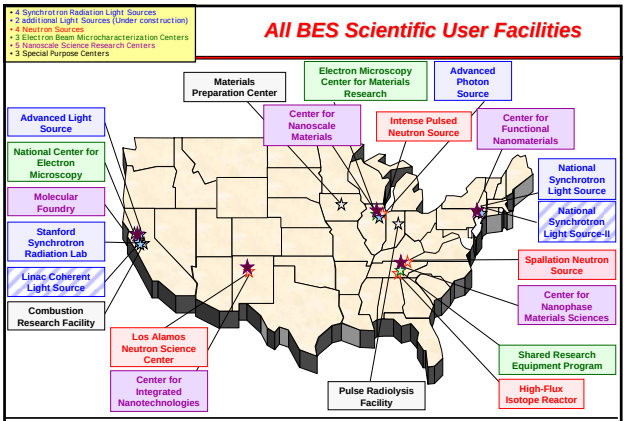
The mission of the Office of Basic Energy Sciences

- Foster and support fundamental research to provide the basis for new, improved, environmentally conscientious energy technologies

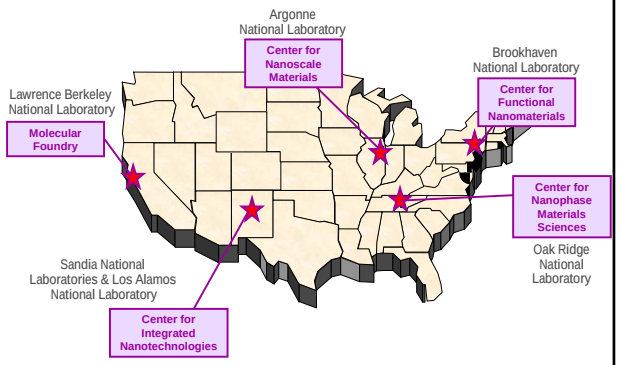


- Plan, construct, and operate major scientific user facilities for "materials sciences and related disciplines" to serve researchers from academia, federal laboratories, and industry

All BES Scientific User Facilities



Five Nanoscale Science Research Centers (NSRCs)



Nanoscale Science Research Centers: Basic Info

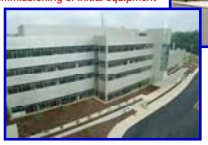
- *Research facilities for synthesis, processing, analysis, and characterization of nanoscale materials*
- *Provide specialized equipment, unique tools, and dedicated scientific and support staff that are difficult for individual institutions to put in place and maintain*
- *Operated as user facilities and available to all. Access determined by peer review of proposals. No cost for precompetitive, non-proprietary work leading to publication; cost recovery for proprietary work.*
- *Co-located at DOE National Laboratories with existing major user facilities (synchrotron radiation light sources, neutron scattering facilities, other specialized facilities) to provide characterization and analysis capabilities*

The five NSRCs are open for business and serving users!

Molecular Foundry (Lawrence Berkeley National Laboratory)

Center for Functional Nanomaterials (Brookhaven National Laboratory) - in process of completing installation, commissioning of initial equipment

Center for Nanoscale Materials (Argonne National Laboratory)

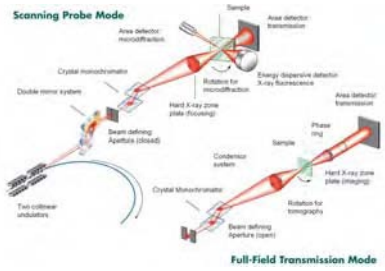


Center for Nanophase Materials Sciences (Oak Ridge National Laboratory)

Center for Integrated Nanotechnologies (Sandia & Los Alamos National Labs)

Unique tools: x-ray synchrotron beamlines with nanoscale resolution

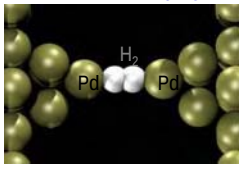
- *Unique instruments to study individual nanostructures*
- *Quantitative structure, strain, orientation imaging*
- *Sensitive trace element and chemical state analysis*



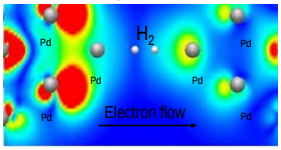
- *Joint nanoprobe effort between CNM and the Advanced Photon Source at Argonne National Laboratory*
- *Similar efforts underway via CFN and the National Synchrotron Light Source at Brookhaven National Laboratory*

Calculating Resistance in the Smallest Possible Junctions

In the ultimate limit, one can imagine a junction across a single molecule. Measurements have been made in recent years on the electron transport through a single molecule of hydrogen, positioned between two metal point contacts via nanofabrication techniques. Now novel calculations have been done to understand the junction resistance. The resistance of the hydrogen molecule is extremely sensitive to the choice of contact material, a trait not seen in macroscopic junctions, and model calculations accurately reproduce these material dependences.



Schematic view of a single hydrogen molecule positioned between two palladium point contacts.

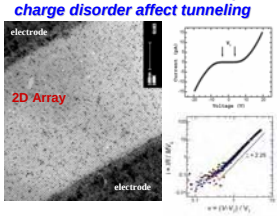


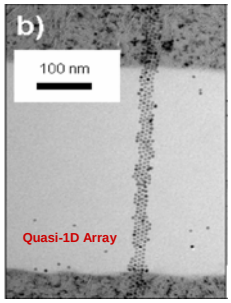
A density plot of the conducting electrons reveals significant build up of electron density (red) behind the tip Pd atom, leading to a high junction resistance and consistent with recent experiments.

Charge Transport in Low-Dimensional Structures: 2D and Quasi-1D Nanocrystal Arrays

Experiments on low-dimensional artificial solids made of nanocrystals have yielded new insights:

- *I-V behavior is highly nonlinear*
- *Threshold voltage scales linearly with array width.*
- *Both structural disorder and quenched charge disorder affect tunneling*

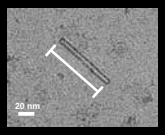




X.-M. Lin, K. Elteto, et al., CNM and U. Chicago

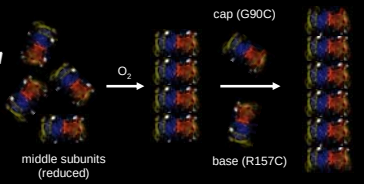
Producing Defined Protein Nanotubes

- *Hcp1 forms a hexameric ring with a large internal diameter*
- *Hcp1 rings stack to form tubes in the crystal lattice*
- *introduction of site-specific modifications stabilizes free-standing tubes*



80 nm (~20 mer)

- *excess of end subunits added*
- *can be selectively modified*
- *length controlled by time, concentration and specificity*



J. Mougous et al., Molecular Foundry and Harvard Medical School

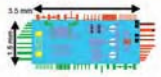
Novel approaches for rapid, reproducible measurements and synthesis

New Tools: Discovery Platforms™

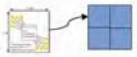
Standardized modular, micro-laboratories—designed and batch fabricated for:

- Integrating nano and micro length scales
- Studying the physical / chemical properties of nanoscale materials and devices
- Directly accessing wide range of CINT external diagnostic and characterization tools

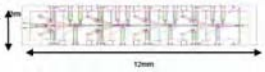
Cantilever Array Platform



Electrical Transport & Optical Spectroscopy Platform



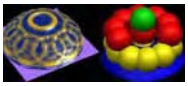
Microfluidic Synthesis Platform



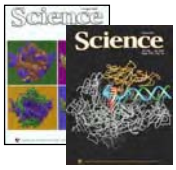
Seeing atoms:

Providing national user facilities for probing materials at the atomic scale

X-ray scattering

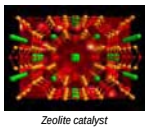


AlNiCo quasicrystal structure

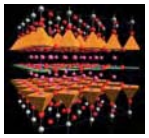


Molecular machines of life

Neutron scattering

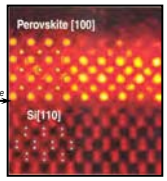


Zeolite catalyst



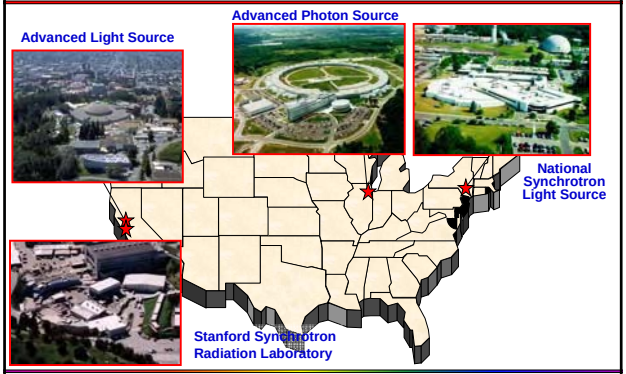
High Tc superconductor

Electron Scattering

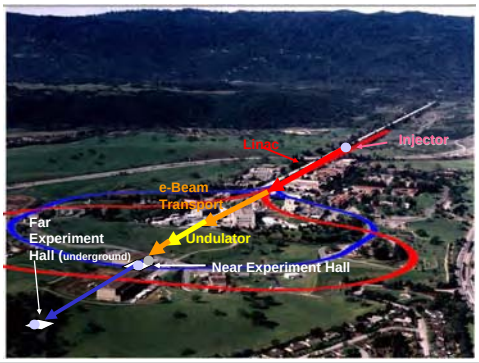


Transmission electron microscope image showing an abrupt interface and low defect density for the ferroelectric $SrTiO_3$ on Si.

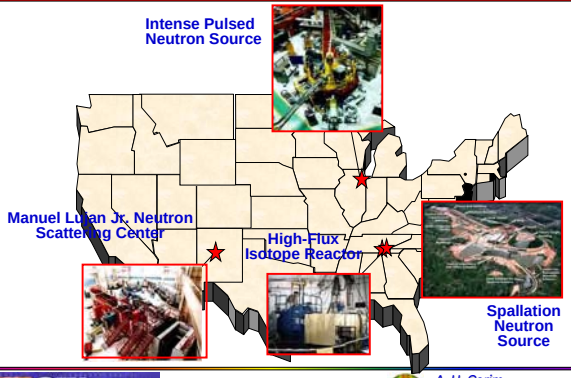
The (existing) BES Light Sources



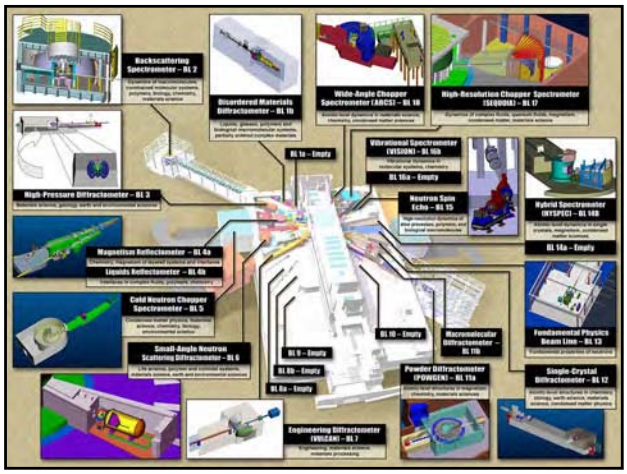
LCLS at SLAC – The World's First X-ray FEL



The DOE-BES Neutron Scattering Centers

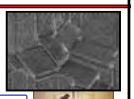


The Spallation Neutron Source

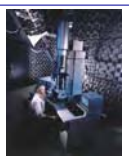


The DOE-BES Electron Scattering User Facilities

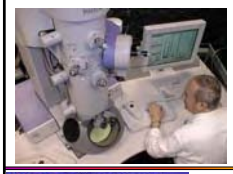
National Center for Electron Microscopy **(NCEM) at Lawrence Berkeley National Laboratory: atomic resolution imaging**



Electron Microscopy Center **(EMC) at Argonne National Laboratory: in-situ studies, including irradiation effects**



Sub-Angstrom Microscopy and Microanalysis building, under construction



Shared Research Equipment **(SHaRE) Program at Oak Ridge National Laboratory: microanalysis and spectroscopy**

For more information:

- ***On DOE's Office of Basic Energy Sciences (BES):***
  http://www.science.doe.gov/bes/

- ***On DOE nanoscience:***
  http://nano.energy.gov

- ***On the DOE-BES Scientific User Facilities:***
  http://www.sc.doe.gov/bes/BESfacilities.htm

NIOSH Nanotechnology Program

Vladimir Murashov
Special Assistant to the Director

National Institute for Occupational Safety and Health
Washington, D.C.

2007 Interagency Workshop on the Environmental Implications of Nanotechnology, September 5, 2007

"The findings and conclusions in this presentation have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be construed to represent any agency determination or policy."

About NIOSH

The National Institute for Occupational Safety and Health is:

the U.S. Federal agency responsible for conducting research and making recommendations for the prevention of work-related injury and illness

NIOSH & Emerging Technologies

OSH Act directs NIOSH to "conduct special research, experiments, and demonstrations relating to occupational safety and health as are necessary to explore new problems, including those created by new technology in occupational safety and health."

29 USC 669 Sec. 20(a)(4)

Concerns Over Nanotechnology Implications

THONG, 2005

Washington Post 2005

Woodrow Wilson Center 2006

NGO Coalition 2007

NIOSH Goals Involving Nanotechnology

- Understand and prevent work-related injuries and illnesses potentially caused by nanoparticles and nanomaterials
- Promote healthy workplaces through interventions, recommendations, and capacity building
- Enhance global workplace safety and health through national and international collaboration on nanotechnology
- Conduct research to prevent work-related injuries by applying nanotechnology products

Understand and prevent work-related injuries and illnesses potentially caused by nanoparticles and nanomaterials

- Toxicology Research
  - Pulmonary effects in mice
  - Nanoparticles enter blood stream
  - Dermal effects
  - Nanoparticle generation system
- Metrology Research
- Control Technology Research
- Exposure Assessment
- Medical Surveillance and Guidance
- Safety Research

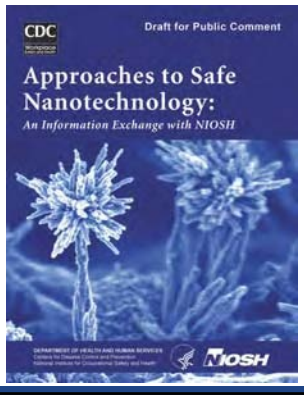
- **Research progress in 10 key areas**
- **Continuing project plans**
- **Opportunities for collaboration**

www.cdc.gov/niosh/topics/nanotech

Promote healthy work places through interventions, recommendations, and capacity building

- NIOSH Field Team
- Approaches to safe nanotechnology: An information exchange with NIOSH
- NIOSH Topic Page
- Nanoparticle Information Library
- National and International Conference

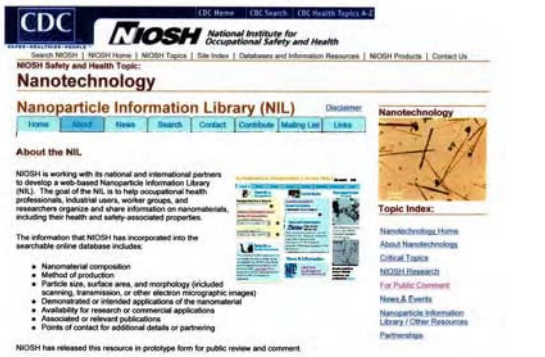
www.cdc.gov/niosh/topics/nanotech



Recommendations from NIOSH

- **Summary of issues**
- **Approaches to consider**
- **Basic Guidance**
- **Updated as new information comes on-line**
- **Input requested**

www.cdc.gov/niosh/topics/nanotech



www.cdc.gov/niosh/topics/nanotech/NIL.html

NIOSH-sponsored conferences

August 29 - September 1, 2007 nano-taiwan.sinica.edu.tw/EHS2007/

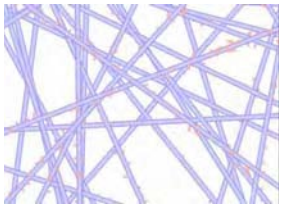
www.ttl.fi/EuroNanOSH

Enhance global workplace safety and health through national and international collaborations on nanotechnology

- Collaborations with various companies (e.g. DuPont, Altairnano, Luna Technologies)
- Participation in inter-agency working groups (NEHI, GIN)
- Participation in ISO TC 229 Nanotechnology Working Group on Health, Safety and Environment
- Collaboration with OECD
- Collaboration with WHO

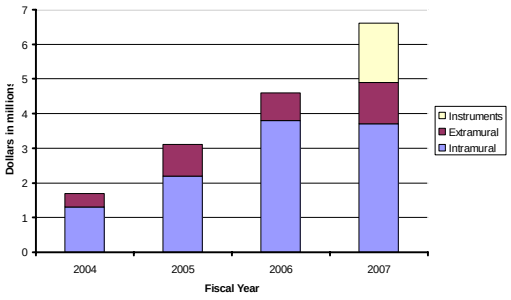
Conduct research to prevent work related injuries by applying nanotechnology products

- Examine applications for filters, sensors, and protective clothing



Electrospun nanofibers, NIOSH

NIOSH Nanotechnology Program Funding



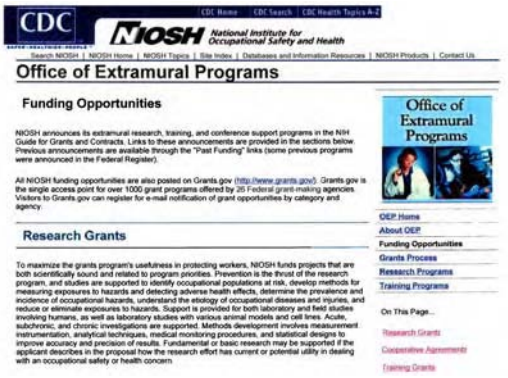
NIOSH Nanotechnology Program Activities In Nanotechnology Research

I. Intramural
   i. National Occupational Research Agenda: Nanotechnology Safety and Health Research Program (2004-2008)
   ii. NIOSH Nanotechnology Research Center (2005-)
   iii. Nanotechnology Research Supplement (2006-2010)
   iv. Nano-Related Division Projects

II. Extramural
   i. Research Grants
   ii. Joint RFAs
   iii. Contracts

www.cdc.gov/niosh/topics/nanotech/strat_plan.html

Extramural Program
http://www.cdc.gov/niosh/oep/



EPA-led Joint Research Solicitation

- Joint Request For Applications with EPA/NCER, NSF and NIH/NIEHS in FY 2004 through FY2006:
  - Nanotechnology Research Grants: Investigating Environmental and Human Health Issues.
- Up to $8 million to support 15-25 research grants and exploratory grants (per year): up to $1 million from NIOSH.
- Focus:
  - research to meet NIOSH mission of providing leadership in preventing work-related illnesses and injuries.

NIH-led Joint Research Solicitation

- Joint Request For Applications with NIH, and EPA/NCER in FY2007:
  - Manufactured Nanomaterials: Physico-chemical Principles of Biocompatibility and Toxicity (R01)
- Up to $4.1 million to support 10-15 research grants and exploratory grants: up to $0.5 million from NIOSH.
- Focus:
  - to identify and investigate the relationships between hazardous working conditions and associated occupational diseases and injuries; to develop more sensitive means of evaluating hazards at work sites, as well as methods for measuring early markers of adverse health effects and injuries; to develop new protective equipment, engineering control technology, and work practices to reduce the risks of occupational hazards; and to evaluate the technical feasibility or application of a new or improved occupational safety and health procedure, method, technique, or system.

Thank you!

Vladimir.Murashov@cdc.hhs.gov

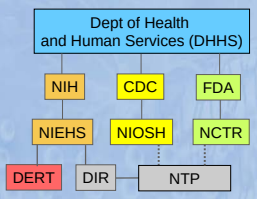
NIEHS activities on Nanotechnology: Nanoscale Science and Toxicology

Nigel Walker Ph.D.
National Toxicology Program
National Institute of Environmental Health Sciences
National Institutes of Health, RTP, NC

Interagency Workshop on the Environmental Implications of Nanotechnology, Washington DC, September 5th 2007

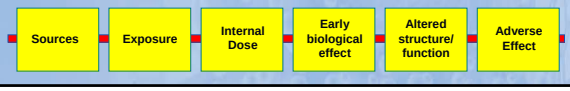
Nano at NIEHS

- Funded by NIEHS
  - Division of Extramural Research and Training (DERT)
    - Grants
    - Training
- Research at NIEHS
  - Division of Intramural Research (DIR)
  - National Toxicology Program (NTP)
    - Contract based research and testing
  - DIR Investigator Initiated
    - Application of nanotechnology in EHS



Areas of emphasis for NIEHS and NTP

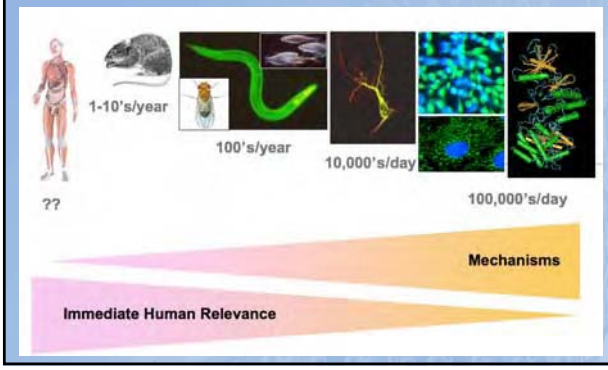
- Exposure and dose metrics
  - How do we measure exposure?
- Internal dose-Pharmacokinetics in biological systems
  - What physiochemical properties determine the absorption, distribution and elimination of nanomaterials?
- Early biological effects and altered structure function
  - What physiochemical properties determine biocompatibility?
- Adverse effects
  - What are the critical determinants of toxicity for those that are toxic?



Experimental Strategies

- Several workshops/reports with common issues/recommendations
  - NTP workshop on Experimental strategies
    - University of Florida-Nov 2004
    - http://ntp.niehs.nih.gov/go/100
  - ILSI-RSI report
    - Oberdorster et al 2005, Particle Fibre Toxicol 2:8
- Current models able to detect manifestations of novel mechanisms of action
  - Use of both in vivo and in vitro approaches
- Need comprehensive physical/chemical characterizations

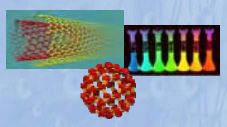
Biological levels and hazard evaluation strategies



Intramural - NTP Nanotechnology Safety Initiative

http://ntp.niehs.nih.gov/go/nanotech

- Scientific Focus
  - Identify key physical-chemical features that govern nanomaterial safety
- Current materials under evaluation
  - Quantum dots
  - Titanium dioxide
  - Carbon fullerenes
  - Nanoscale silver
  - Multi-walled carbon nanotubes
  - Nanoscale gold
  - Dendrimers



- Contact: Nigel Walker, walker3@niehs.nih.gov

Extramural - Enabling technologies

- Environmental Sensors
  - Deployable sensor devices for a broad range of environmental exposures
- Biological Sensors
  - Develop and apply technologies to link exposure with disease etiology
- Intervention devices
  - Drug delivery devices and therapeutic nanoscale materials
- Remediation devices
  - Primary disease prevention through the elimination of exposure
    - Catalysis or chelation
- Contact: David Balshaw: balshaw@niehs.nih.gov

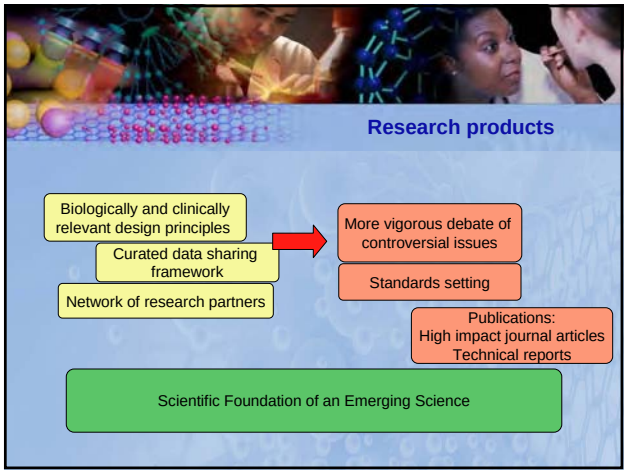
Extramural - Fundamentals of Biological Response

- FY06-Human Health Effects of Manufactured Nanomaterials
  - Joint solicitation between EPA, NSF, NIOSH, NIEHS/NIH
  - Funded three applications $400K /year for 3 years
    - Transmembrane transport, cardiovascular toxicity and oxidative stress
- FY07-Manufactured Nanomaterials: Physico-chemical Principles of Biocompatibility and Toxicity
  - NIEHS lead with additional partners
    - NCI,NEI, NHGRI, NIDCR, NIGMS, and EPA, NIOSH
  - Review process completed and approx 10 grants may be funded
- Contact: Sri Nadadur, nadadurs@niehs.nih.gov

Taking the Next Step:

- **Building on the NIEHS investment and core competencies**
- **Partnering for integrated research success**
- **Consistent with US goals for safe commercialization and innovation**

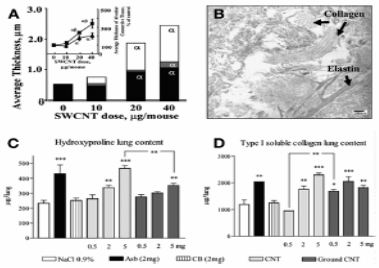
- Scope
  - Examine the fundamental physicochemical interactions of ENM with biological systems at the molecular, cellular, and organ level, as well as associated pathophysiologic processes
- Rationale
  - New knowledge of molecular, cellular, and organ system biology and identify clinically relevant properties of ENM
  - critical for design of ENM with maximum human and environmental biocompatibility and safety
- Contact: Sally Tinkle, stinkle@niehs.nih.gov



Pulmonary and Systemic Biocompatability of Inhaled Carbon Nanotubes

Jake McDonald
Lovelace Respiratory Research Institute
Albuquerque, NM

Instillation of CNT Resulted in Significant Lung Tissue Damage



Lam:All CNT showed lesions/granulomas after 2- 5 mg/kg dose
Warheit: No dose or time dependence to pathology or inflammation

Hypothesis: Inhaled Carbon Nanotubes will Not Cause Pulmonary Injury or Inflammation after High Dose Exposures

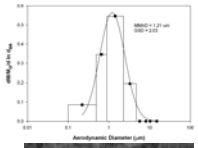
LRRI Inhalation Exposure System for CNT

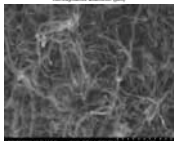
- **Obtained Aligned MWCNT from NTP (Shenzhen, China)**
  - Purity >95%
  - Diameter 10-20 nm
  - Length 5-10 µm
  - Amorphous carbon < 3%
  - Ash (catalyst residue) < 0.2%
  - Special surface area 40-300 m2/g (actual 100 m2/g)
- **Aerosolized with jet-o-mizer followed by cyclone**


www.mefs.co.jp

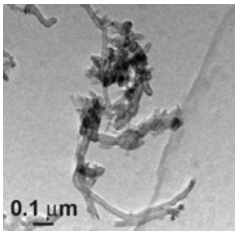
Characterization of Bulk and Aerosol Composition Key Component of Work

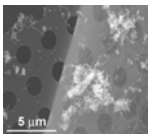
- **TEM, HR-TEM**
- **SEM**
- **XRD**
- **X-Ray Photoemission Spectroscopy**
- **Raman**
- **Impactors (aerosol only)**
- **Differential Mobility (aerosol only)**
- **Mass Spectrometry**
- **BET (gas adsorption) for Surface Area**

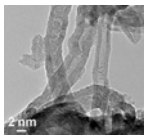




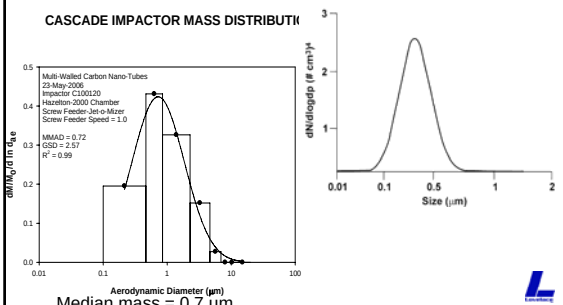
Aerosolized MWCNT







Particle Mass and Number Size Distribution

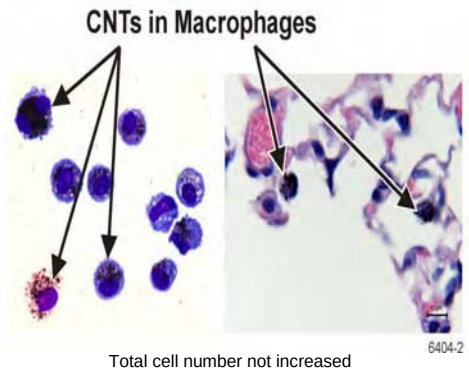


Median mass = 0.7 µm

Study Design

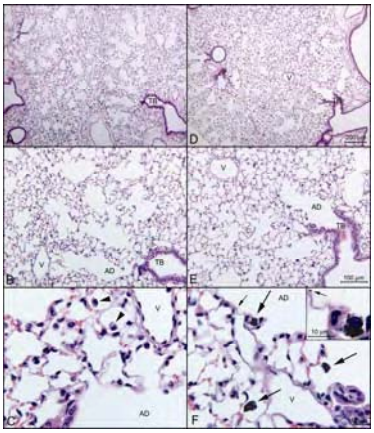
- **Male C57Bl/6 mice**
  - **6 hr/day, 7 days/week**
  - **Sham, 300, 1000, 5000 µg/m³ (0.2-10 µg deposited/day)**
  - **Sacrifice at 7 and 14 days**
    - **Lavage cell counts and biomarkers**
    - **Lung biomarkers**
    - **Lung pathology**
    - **Immune Function-Spleen**
    - **Gene upregulation: lung, spleen**

CNTs in Macrophages

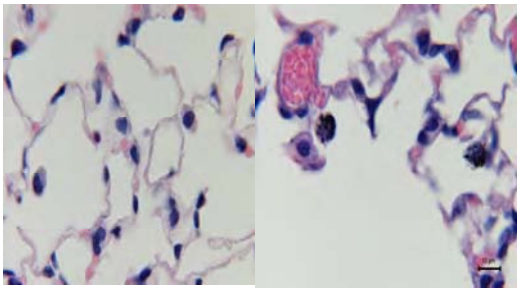


Total cell number not increased
No increase in neutrophils, lymphocytes, etc

Control 5mg/m³



Clean Air 40 X Showing Alveoli and Macrophages

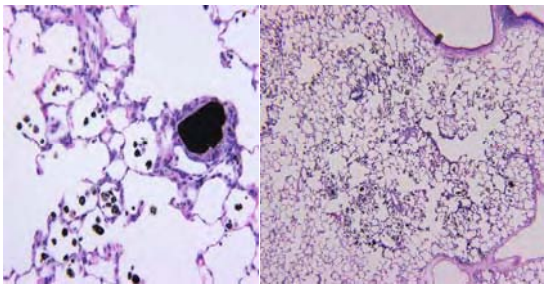


Summary and Future Directions

Inhaled MWCNT showed unremarkable pulmonary inflammation and pathology at high doses and by inhalation.

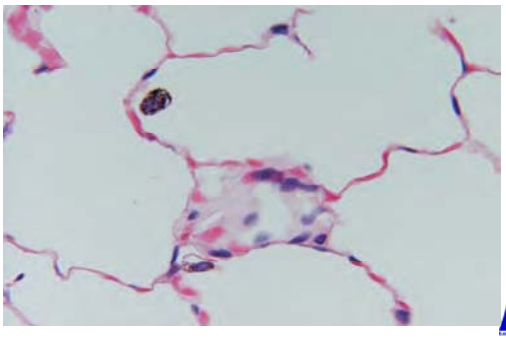
- **Contradicts initial hypothesis, which was based on marked pulmonary effects reported by others after instillation or aspiration**
  - **Dose?**
  - **Route of Administration?**
  - **SWCNT vs MWCNT vs ?**
  - **Time?**

Instilled Diesel Soot at ~2 mg/kg Results in Granulama formation, inflammatory cell infiltration, change in inflammatory mediators, etc



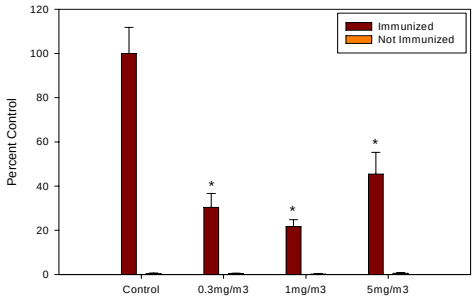
10x 4x

Diesel Engine Exhaust Inhalation at 1 mg/m^3 Resulted in Little to No Pulmonary Findings Except Particulate in Macrophages

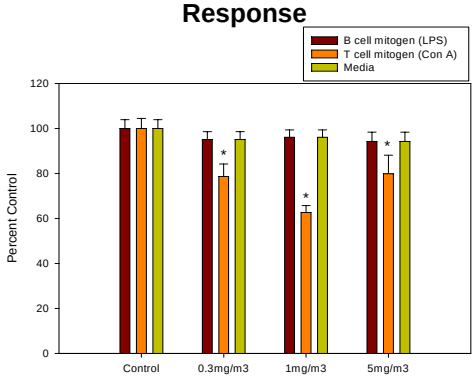


Systemic Response to Inhaled MWCNT

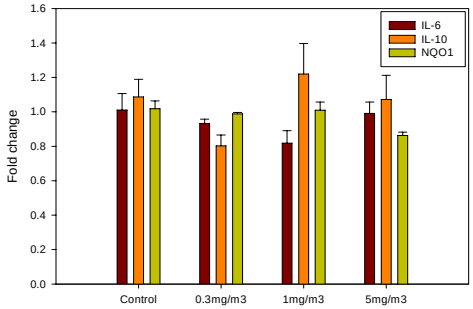
Suppressed Antibody Response



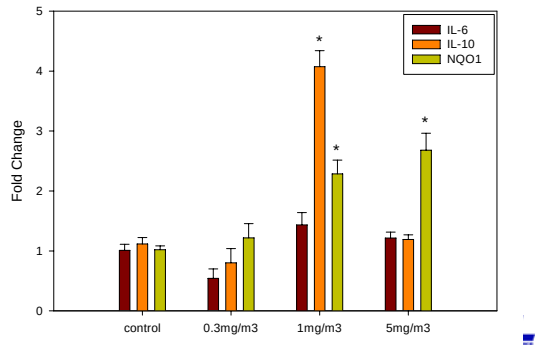
Suppressed T cell mitogen Response



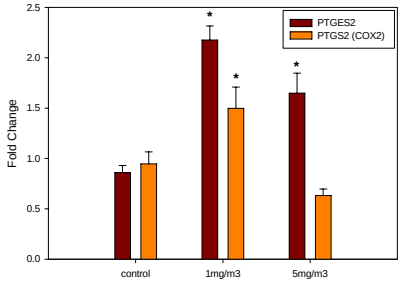
Gene Expression unaltered in lung tissue

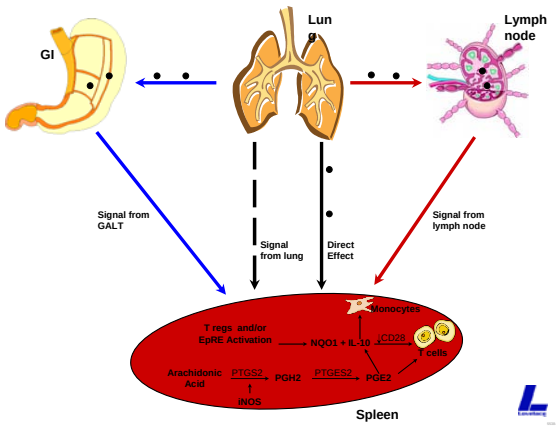


Gene Expression in Spleen



Prostaglandin Associated Enzymes





Important Considerations

- We have observed systemic immunosuppression with inhalation exposures in the past (Diesel, woodsmoke, coal)
- Often times no pulmonary effects accompanied these systemic immune function changes
- The immune responses shown here are likely NOT unique to MWCNT, but this has not been proven

Thanks

- Collaborators
  - Randy Vander Wal (USRA-NASA)
  - Scott Burchiel
  - JeanClare Seagrave
  - Leah Mitchell
  - Andrew Giggliotti
  - Chemistry/Exposure Staff
  - Necropsy Staff
- Funding
  - EPA, NIEHS

Metal Nanoparticle Tissue Distribution following *In Vivo* Exposures

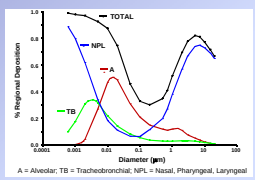
EPA Nanograntees Meeting, Sept. 5-7, 2007

Interagency Workshop on the Environmental Implications of Nanotechnology

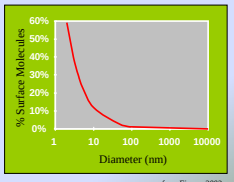
Alison Elder
Department of Environmental Medicine
University of Rochester

Introduction

- Studies with ultrafine particles have demonstrated extrapulmonary translocation.
- What properties affect the tissue distribution of nanosized particles?



A = Alveolar; TB = Tracheobronchial; NPL = Nasal, Pharyngeal, Laryngeal



from Fissan, 2003

Hypothesis

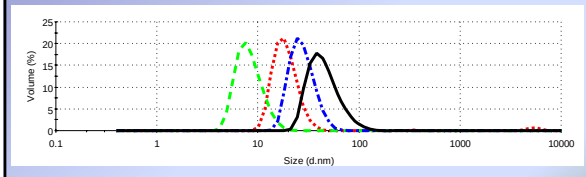
The tissue distribution of nanomaterials following respiratory tract or systemic exposure is a function of their surface properties.

Methods:
Nanoparticle Characteristics

- Characteristics of the QDots:
  - CdSe/ZnS core-shell particles coated with polymer, ~5 nm core-shell diameter (Invitrogen) – 565 nm emitters
  - PEG, PEG-amine, or carboxyl conjugated surfaces
  - Hydrodynamic radii:
    - 14 (carboxyl), 15 (PEG-amine), 35 (PEG) nm – reported *(Ryman-Rasmussen et al., 2006)*
    - 13 (carboxyl), 17 (PEG-amine), 23 (PEG) nm – in saline
  - Zeta potentials (in 0.9% saline, pH 7.4):
    - -40.0 (carboxyl), -0.3 (PEG-amine), -1.5 mV (PEG)

Methods:
Nanoparticle Characteristics

- Characteristics of colloidal Au particles:
  - 5 nm primary particle size (Ted Pella, Inc.)
  - Coated with albumin, 5 kDa PEG, or 20 kDa PEG



Surface Coating	Peak Mean
Citrated Au	8 nm
RSA-Au	19 nm
PEG (5K)-Au	28 nm
PEG (20K)-Au	47 nm

from: Dr. A. Rinderknecht

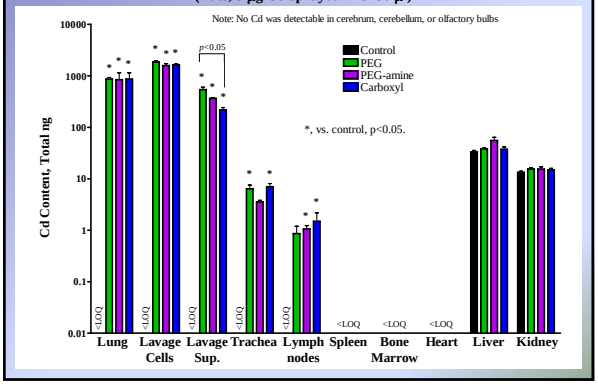
Methods:
Nanoparticle Exposures

- Exposures to QDots (dose expressed as Cd content):
  - Intratracheal microspray (5 μg Cd/150 μl saline)
  - Intravenous injection (1.7 μg Cd/200 μl saline)
- Exposures to colloidal Au:
  - Intratracheal microspray (50 μg/150 μl saline)
  - Intravenous injection (15 μg/200 μl saline)

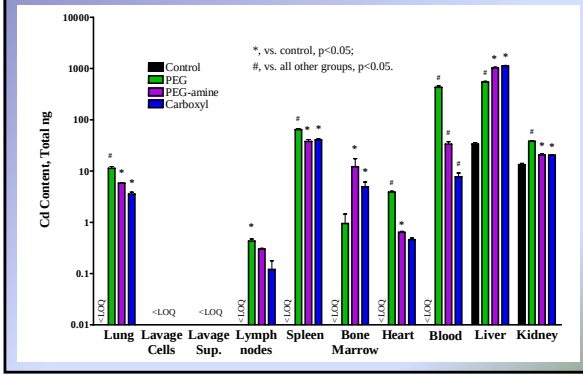
Lung Inflammation following NP Exposure?

- Inflammation (as determined by percentage of lavage fluid PMNs) can significantly alter the translocation of nanoparticles from the lung to the blood and vice versa *(e.g. Heckel et al., 2004)*:
  - **QDots**: no significant increases from controls;
  - **Colloidal Au**: PEGylated particles caused significant increases in PMNs (10-13%) when delivered via ITM; not affected by other coatings, route of exposure.

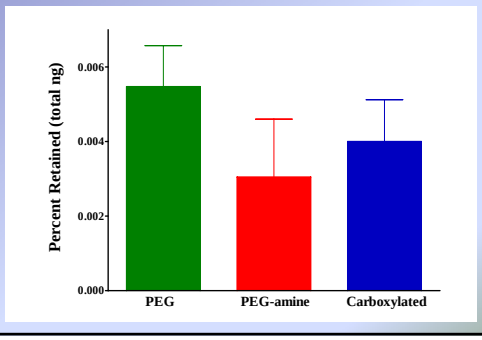
Tissue Cadmium Content 24 hrs following Intratracheal Microspray Exposure of Surface-Modified QDots (Rats, 5 μg Cd sprayed in 3x50 μl)



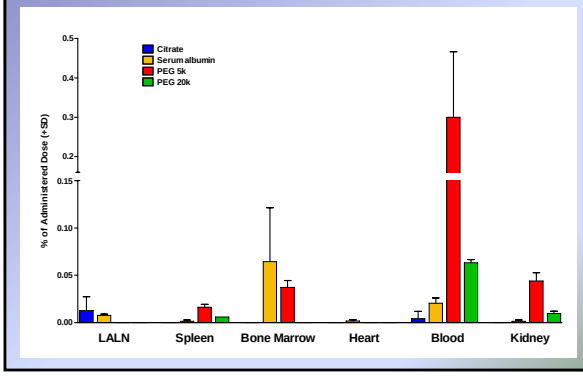
Tissue Cadmium Content 24 hrs following Intravenous Exposure to Surface-Modified QDots (Rats, 1.7 μg Cd injected in 200 μl)



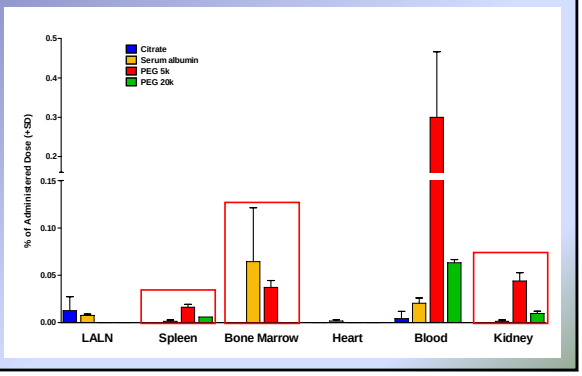
Retention of Cd in Olfactory Bulb following Intranasal Instillation of QDots (~4 μg) into Left Naris (right olfactory bulb background-corrected total tissue Cd)



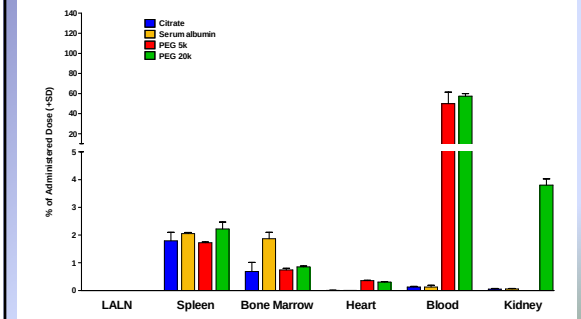
Tissue Au Content 24 hrs following Intratracheal Microspray Exposure to Colloidal Au Nanoparticles with Different Surface Coatings



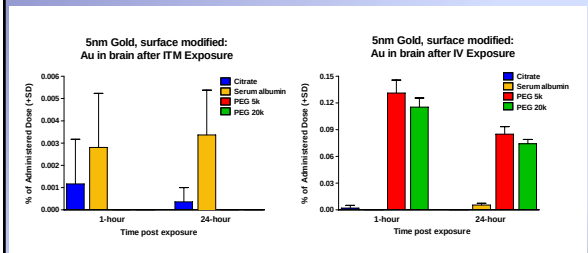
Tissue Au Content 24 hrs following Intratracheal Microspray Exposure to Colloidal Au Nanoparticles with Different Surface Coatings



Tissue Au Content 24 hrs following Intravenous Exposure to Colloidal Au Nanoparticles with Different Surface Coatings



Translocation of Nanogold to the Brain



Summary of Results

1. Nanoparticles delivered via the lower respiratory tract are translocated to extrapulmonary tissues
   - Dependent on particle physicochemical characteristics.
2. Nanoparticles can be retained in small amounts by the brain following a single exposure
   - Dependent on particle physicochemical characteristics and portal of entry.

Remaining Questions

Short-term:

- More thoroughly evaluate kinetics of nanoparticle translocation;
- Where are the nanoparticles localized (which cells, what subcellular structures?)?

Long-term:

- Characterize translocation to the CNS as a function of the particle surface and its interactions with endogenous proteins;
- Characterize elimination of nanoparticles from the CNS.

Acknowledgements

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Pratim Biswas

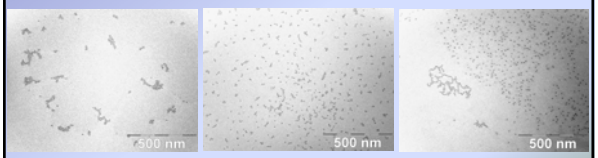
Grant Support:

EPA
DoD, NSF

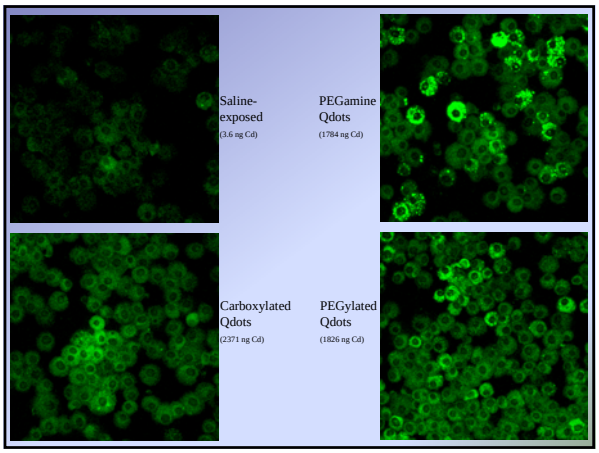
Hypothesis

The tissue distribution of nanomaterials following respiratory tract or systemic exposure is a function of their surface properties.

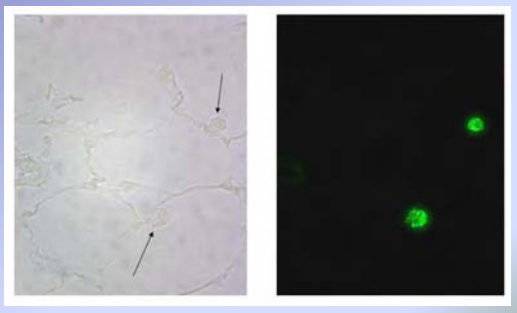
CdSe-ZnS Quantum Dots in 0.9% Saline

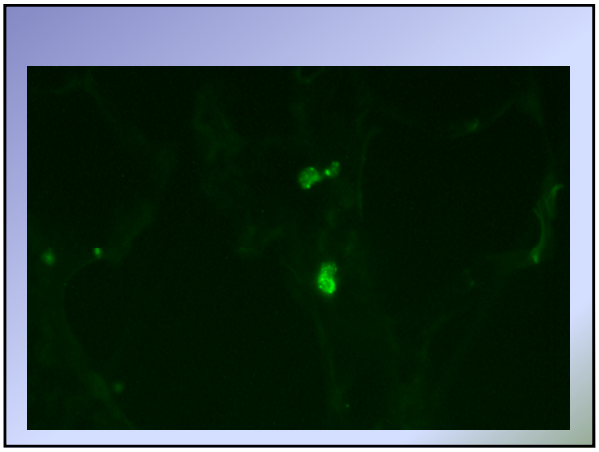


PEGylated 23 nm | **PEGamine 17 nm** | **Carboxylated 13 nm**



Carboxylated QDots in Rat Lung Tissue



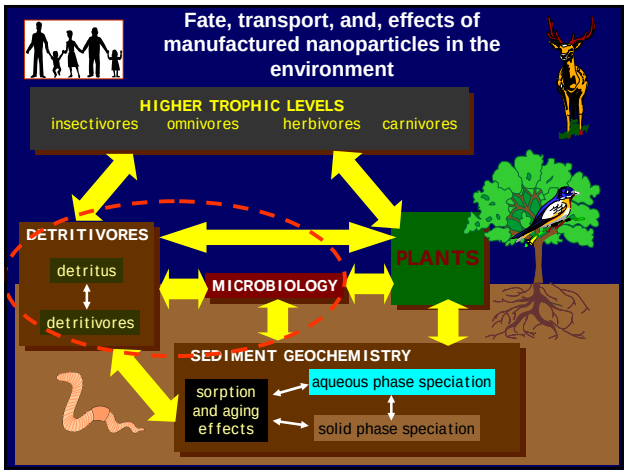


Bioavailability, Toxicity, and Trophic Transfer of Manufactured ZnO Nanoparticles: A view from the bottom

PI: Paul M. Bertsch
Co-PIs: Travis Glenn, Andrew L. Neal, Phillip Williams, Brian P. Jackson-Dartmouth College
Collaborator: Jason M. Unrine, Pamela J. Morris -MUSC
Post doc: Nadine J. Kabengi
Ph.D. students: Hongbo Ma & Benjamin A. Neely-MUSC

The University of Georgia
Savannah River Ecology Laboratory

This research is funded by U.S.EPA-Science To Achieve Results (STAR) Program
Grant # RD 832530



OBJECTIVES to evaluate:

One: the bioavailability and toxicity of manufactured nanoparticles (ZnO-np) as a function of particle size to model soil bacteria (*Burkholderia vietnamiensis* and *Cupriavidus necator)* & the model detritivore *Caenorhabditis elegans* as referenced against aqueous Zn^{2+}.

Two: the ability of manufactured ZnO-np to be transferred from one trophic level to the next as assessed in the simple food chain consisting of pre-exposed *B. vietnamiensis* & *C. elegans*

Three: the additive, synergistic or antagonistic effects of manufactured ZnO-np on the toxicity of Cu^{2+} to *B. vietnamiensis* and *C. elegans.*

HYPOTHESES

1: The bioavailability and toxicity of manufactured ZnO-np increases with decreasing particle size (i.e. 6 nm vs. 80 nm)

2: The toxicity of ZnO-np to model soil bacteria and *C. elegans* is lower than an equivalent concentration of dissolved Zn^{2+}

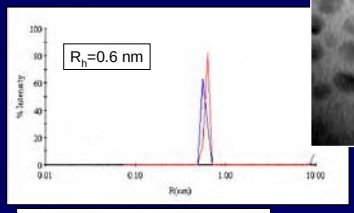
3: The bioavailability and toxicity of ZnO-np introduced via trophic transfer differs from direct exposure

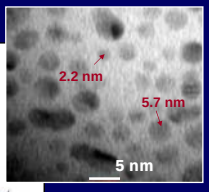
4: ZnO-np alter the bioavailability and toxicity of dissolved metals

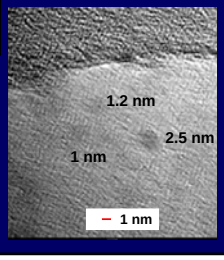
Characterization: lot to lot variability

	Lot 1	Lot 2
pH	6.25	4.5
[Zn] g L^{-1}	56.0	72.0
[Zn] moles L^{-1}	0.86	1.11
[Acetate] moles L^{-1}	2.33 M	3.08 M
PZNC	ND	pH 6-7

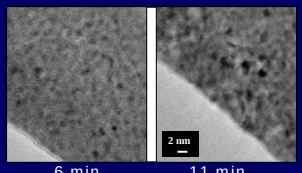
Characterization: size issue





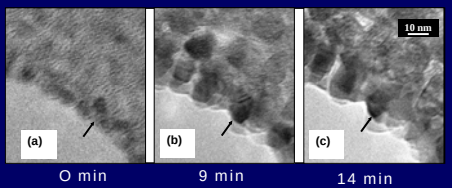


Characterization: TEM artifacts

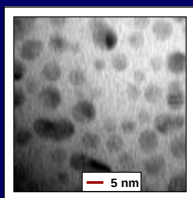


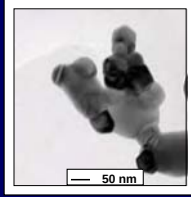
At room temperature

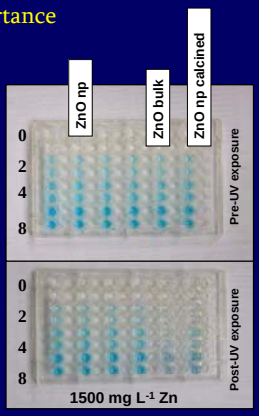
At 200°C



Characterization: Acetate Importance

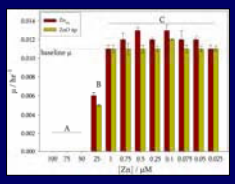


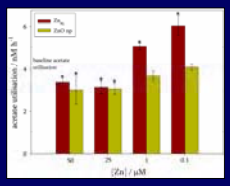




Nanoparticle-Bacteria interaction

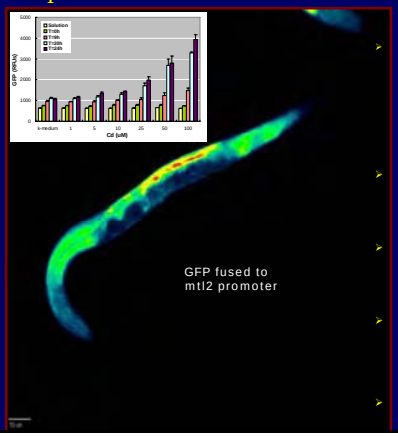
Cupriavidus necator





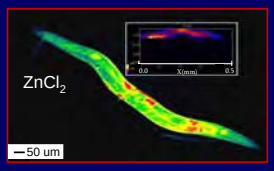
- No significant difference between $Zn^{2+}_{aq.}$ and ZnO-np growth rates
- Higher OAc utilization rates with $Zn^{2+}_{aq.}$ compared to ZnO-np
- Evidence for bioavailability of Zn ion, but not ZnO-np
- Epifluorescence microscopy indicates an increased number of cells with compromised membranes associated with ZnO-np vs. free ion

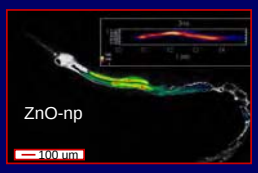
Nanoparticle-detritivore interactions



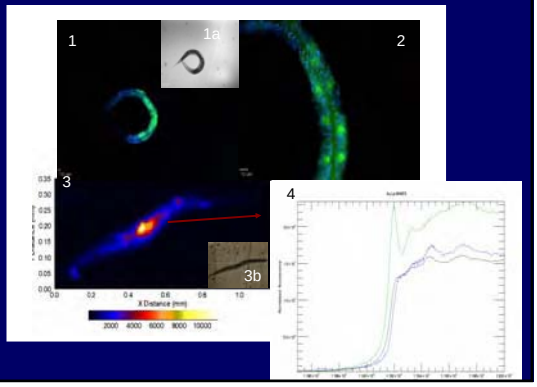
- *Caenorhabditis elegans* grown on solid k-medium (KCl, NaCl, CaCl, $MgSO_4$, peptone, cholesterol) with a lawn of *Escherichia coli* OP-50
- exposures in aqueous medium of either DDI or k-medium
- Simple organism (approx. 959 cells), genome sequenced
- Feeds on bacteria and other particles < 5 µm in diameter
- GFP induction shows time and metal concentration-dependence.
- Acetate is not toxic to *C. elegans*

Zn spatial distribution & *mtl2::GFP* expression in exposed nematodes:

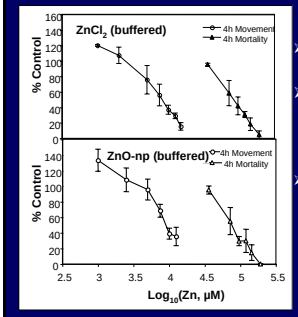




Uptake of nanoscale Au in *C. elegans* (mtl2::GFP)

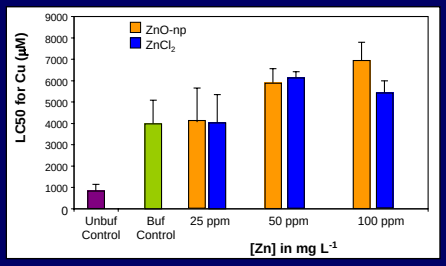


Behavior & Lethality data for $ZnCl_2$ and ZnO-np

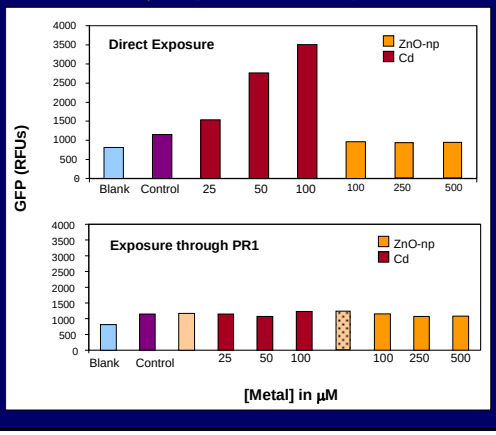


- $ZnCl_2$: EC50= 8.54mM (95% CI: 6.82-10.69mM) LC50=83.1(+19.2)mM
- ZnO-np: EC50= 9.42mM (95% CI: 8.09-10.97mM) LC50=79.1(+16.8)mM
- No significant difference in EC50 or LC50 was found between $ZnCl_2$ and ZnO-np in buffered medium (acetate buffered, pH=6.0).

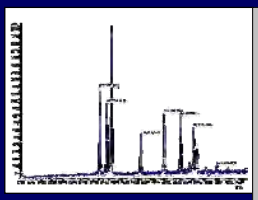
Effects of ZnO-np and $ZnCl_2$ on Cu toxicity



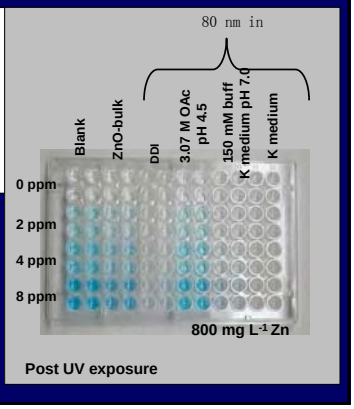
Preliminary trophic transfer experiment



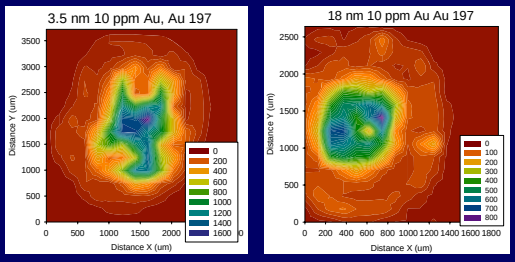
Initiation of experiments with 80 nm ZnO-np



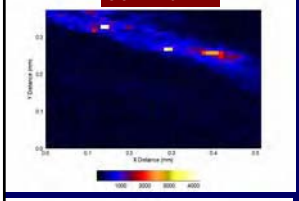
Surface area
BET = 17.04 $m^2\ g^{-1}$

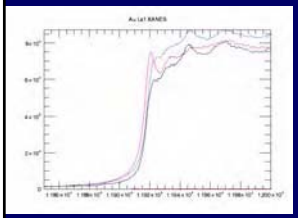


Uptake of nanoscale Au in *Eisenia fetida: size dependence*



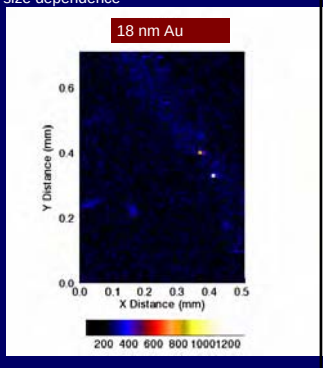
3.5 nm Au





Uptake of nanoscale Au in *Nicotiana xanthi:* size dependence

18 nm Au



Summary

Characterization

- Size determination is a critical issue
- TEM may not be the best method for size determination of ZnO
- Acetate controls ZnO-np reactivity, passivates surface sites
- Removal of acetate leads to flocculation/ aggregation of ZnO-np primary particles but promotes surface reactivity

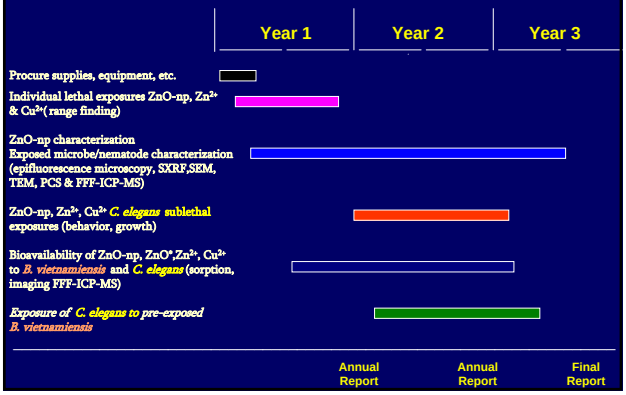
Bacteria

- No difference in growth rate between ZnO-np & Zn^{2+}(aq)
- Higher OAc utilization rates with Zn^{2+}_{aq} compared to ZnO-np
- Evidence for Zn bioavailability from Zn ion, but not ZnO-np
- Cells with compromised membranes associated with ZnO-np compared to free ion
- Possible different mechanism of toxicity

Nematodes

- ZnO-np LC_{50}/EC_{50} not significantly different from $Zn^{2+}_{(aq)}$
- Different mechanism of toxicity
- At [Zn]>100 $mg.L^{-1}$, ZnO-np decreases Cu toxicity as compared to Zn^{2+}(aq)
- No significant GFP was induced either in 100uM Cd or 500uM ZnO-np through PR1 exposure.

Project Schedule



Manuscripts in preparation

A.L. Neal, N. J. Kabengi, and P.M.Bertsch. Toxicity of Zinc Oxides Nanoparticles and Aqueous Zinc to the Soil bacterium *Cupriavidus necator.*

N.J. Kabengi, J.M. Unrine, A.L. Neal, and P.M. Bertsch. Characterization and surface-reactivity of commercially manufactured ZnO nanoparticles: The importance of the acetate counter-anion.

N.J. Kabengi, S. Wu, J. Shields, P.M. Bertsch. 2007. In situ investigation of Zinc oxide nanoparticle growth by transmission electron microscopy: implications for size determination.

H. Ma, J.M. Unrine, A.L Neal, P.L Williams, P.M Bertsch. Bioavailability and toxicity of nano-sized ZnO in the nematode *Caenorhabditis elegans*.

B.A Neely, A. G. Sutter, D. W. Bearden, P. M. Bertsch, and P. J. Morris. Microbial Growth Affects Zinc Oxide Nanoparticle Structure and Toxicity.

Future Work

Results from year 1 and 2 provide a framework for directing the third year of the project

- Characterization work of 80 nm size ZnO nanoparticles
  - Under various chemical conditions
- Bioavailability/toxicity/behavior studies with 80 nm size ZnO nanoparticles
- Continue exposure experiments with Cu^{2+}
- Bioavailability/Toxicity studies
  - Differences in toxicity mechanisms of $ZnCl_2$ and ZnO-np to *B. vietnamensis, C. necator*, and *C. elegans*
  - Bioavailabilty and toxicty of ZnO-np introduced via trophic transfer as opposed to direct exposure.
  - Identify chemical speciation of Zn in concentrated regions in tissues
  - Examine potential transformation of ingested ZnO-np

Biochemical, Molecular and Cellular responses of zebrafish exposed to metallic nanoparticles

David Barber
University of Florida
Center for Environmental and Human Toxicology

Goals

- Expand database of acute toxicity of metallic nanomaterials in aquatic organisms
- Evaluate role of particle composition and dissolution in gill toxicity
- Determine role of particle surface charge in uptake and retention of nanomaterials in aquatic organisms

Acute toxicity of nanometallics: Zebrafish adults

Metal	48 hr Soluble LC_{50}	48 hr Nano-particle LC_{50}
Aluminum	7.92 mg/L	>12.5 mg/L
Copper	0.13 mg/L	**0.94 mg/L**
Silver	22.5 ug/L	**7.1 mg/L**
Cobalt	> 10 mg/L	> 10 mg/L
Titanium dioxide	> 10 mg/L	> 10 mg/L
Nickel	> 10 mg/L	> 10 mg/L

Acute toxicity of nanometallics: Zebrafish fry

Metal	48 hr Soluble LC_{50}	48 hr Nano-particle LC_{50}
Aluminum	> 10 mg/L	>12.5 mg/L
Copper	1.78 mg/L	**0.71 mg/L**
Silver	10 ug/L	6.3 mg/L
Cobalt	> 10 mg/L	> 10 mg/L
Titanium dioxide	> 10 mg/L	> 10 mg/L
Nickel	> 10 mg/L	> 10 mg/L

Acute toxicity of nanometallics: *Daphnia pulex*

Metal	48 hr Soluble LC_{50}	48 hr Nano-particle LC_{50}
Aluminum	> 10 mg/L	3.65 mg/L
Copper	8.68 ug/L	**12.5 ug/L**
Silver	0.85 ug/L	**41.7 ug/L**
Cobalt	9.7 mg/L	27.8 mg/L
Titanium dioxide	> 10 mg/L	> 10 mg/L
Nickel	1.48 mg/L	3.8 mg/L

Acute toxicity of nanometallics: *Ceriodaphnia dubia* neonates

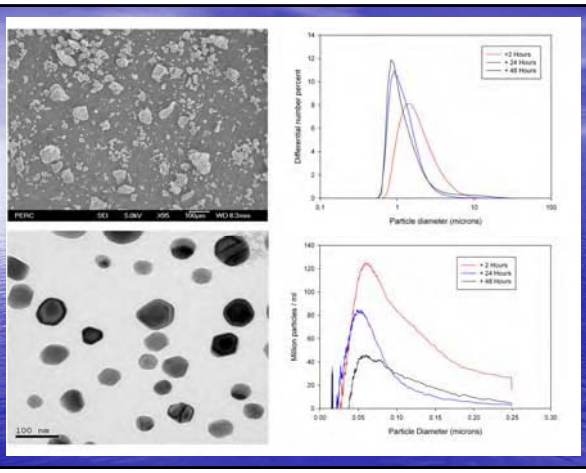
Metal	**48 hr soluble LC50**	**48 hr particle LC_{50}**
Aluminum	153 µg/L	3.99 mg/L
Copper	0.58 µg/L	0.42 mg/L
Silver	0.16 µg/L	0.07 mg/L
Cobalt	94.7 µg/L	1.67 mg/L
Nickel	19.7 µg/L	0.67 mg/L

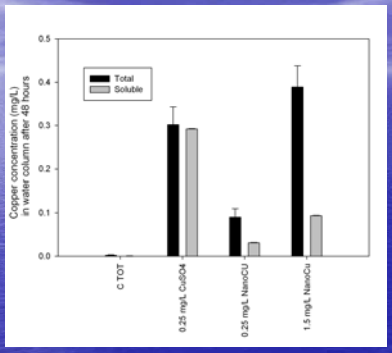
Acute toxicity of nanometallics: inhibition of algae growth

Metal	96 hr Nano-particle IC_{50}
Aluminum	8.28 mg/L
Copper	0.54 mg/L
Silver	0.19 mg/L
Nickel	0.35 mg/L

Particle Dosimetry

	LC_{50} expressed as particle number (particles/L) and surface area (m^2/L)		
Species	Copper	Silver	Nickel
D. pulex	1.03E+11 (2.02E-4)	5.83E+10 (1.14E-4)	6.67E+12 1.31E-2)
C. dubia	7.18 E+11 (1.41E-3)	9.76E+10 (1.92E-4)	1.16E+12 (2.27E-3)
D. rerio (adult)	1.61E+12 (3.16 E-3)	1.03E+13 (2.02E-2)	>1.72E+13 >(3.37E-2)
D. rerio (larval)	1.22E+12 (2.39E-3)	1.05E+13 (2.06E-2)	>1.72E+13 >(3.37E-2)

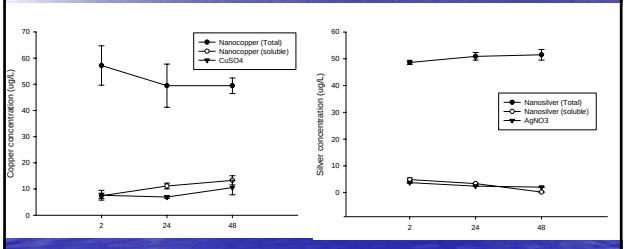




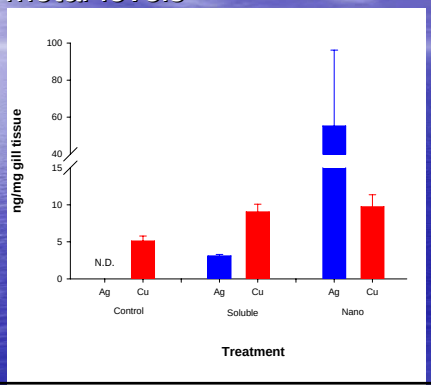
Evaluate role of particle composition and dissolution in gill toxicity of metallic particles

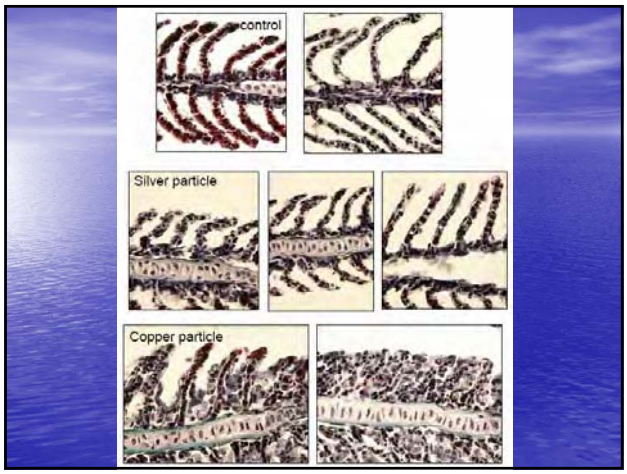
- Experiments examined TiO_2, silver and copper particles
- Dosed at ~NOEC concentrations for particles (100ug/L for Cu, 1000 ug/L for Ag) or concentration of soluble metal released by particles
- Evaluated gill metal uptake, histology and transcriptional changes at 24 and 48 hours

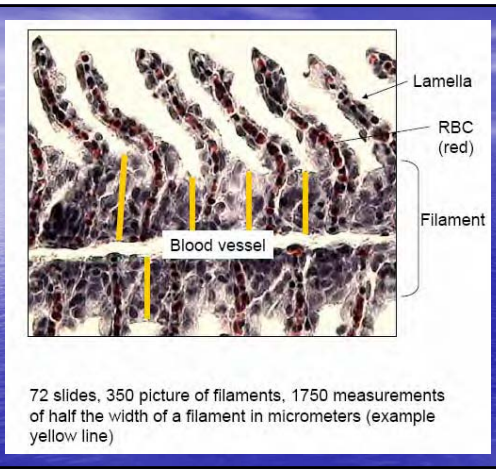
Total and soluble metals during exposures



Particle exposure leads to elevated gill metal levels

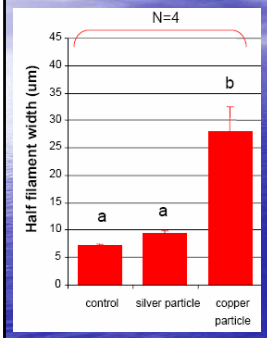


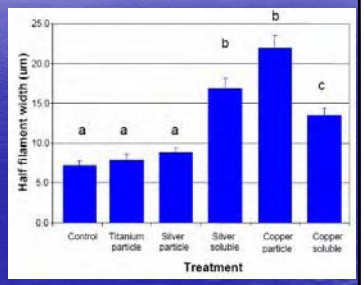




72 slides, 350 picture of filaments, 1750 measurements of half the width of a filament in micrometers (example yellow line)

Gill Filament Thickening



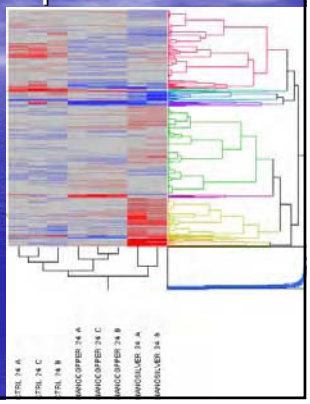


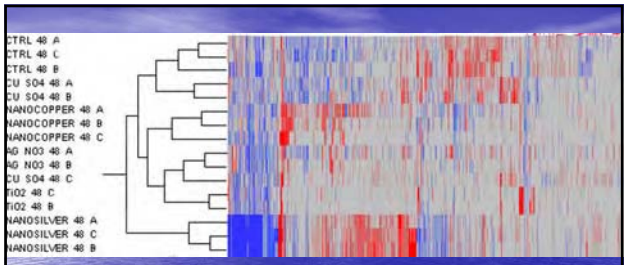
24 hours

48 hours

Transcriptional Responses of Gill

- 3 arrays/treatment
- Analyzed with reference design
- Transcriptional responses at equi-toxic concentrations are markedly different between particles

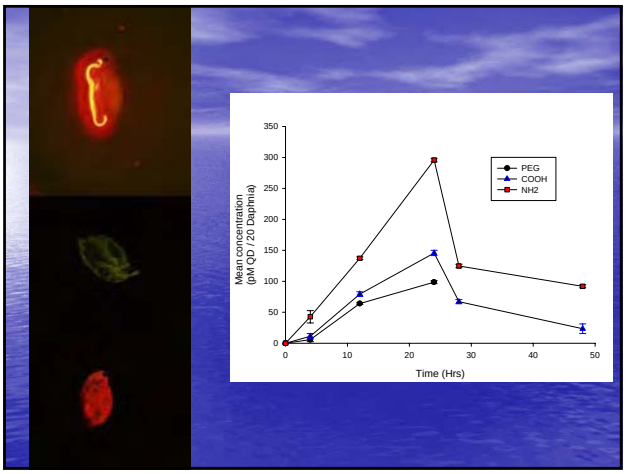




- Particles are different from soluble forms of metals at equal concentrations of soluble material
- TiO2 produces some unique responses, though does not cause overt toxicity
- Particle responses are time dependent

Determine role of particle surface charge in uptake and retention of nanomaterials in aquatic organisms

- Examined uptake and retention of PEG, NH2, and COOH quantum dots in daphnia



Conclusions

- Nanometals can be acutely toxic to aquatic organisms, though typically less toxic than soluble counterparts
  - Particularly to filter-feeders, where LC50s are measured in ug/L
- Nanoparticles aggregate rapidly once introduced into water, but large numbers of nanosized particles are likely to remain in the water column for long periods of time
- Change in particle exposure with time makes dosimetry problematic
- Effects of some nanometals are incompletely explained by dissolution
  - Effects appear to depend on particle composition and are not generic responses
- Charge influences uptake of nanomaterials in daphnia
- Future work will focus on mechanisms

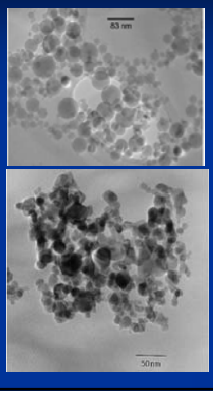
Collaborators

- Center for Environmental and Human Toxicology
  - Nancy Denslow
  - Joe Griffitt
  - Jing Luo
  - Roxana Weil
- Particle Engineering Research Center
  - Dr. Kevin Powers
- Department of Zoology
  - Dr. David Evans
  - Kelly Hyndman
- Department of Environmental Engineering Sciences
  - Dr. Jean-Claude Bonzongo
  - Jie Gao
- Funding from the National Science Foundation (BES054920)

Acute and Developmental Toxicity of Metal Oxide Nanoparticles in Fish and Frogs

George Cobb and Shawna Nations
Texas Tech University
Christopher Theodorakis
Southern Illinois University
Elizabeth Carraway and Xin Xu
Clemson University

Metal Oxide Nanoparticles



- Catalysts
- UV protectants (ZnO, TiO_2)
- Wood preservation
- Marine antifoulants
- Deodorants
- Polishing agents
  - Glass
  - Dental
  - Semiconductors
- Antimicrobial
  - Textiles
  - Foot powder
  - Coatings

Objectives

- Determine the environmental hazard of Fe_2O_3, ZnO, CuO, and TiO_2
- Evaluate acute and chronic toxicity
- Fathead minnows (*Pimephase promelas*) and African clawed frog (*Xenopus laevis*)

Hypothesis

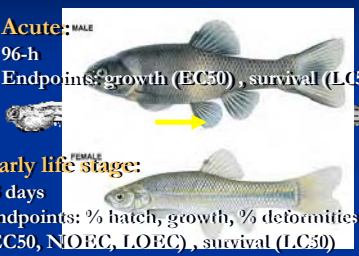
- Nanoparticle exposure will affect the survival, growth, development, egg hatchability, and metamorphosis of these organisms

Approach

Flow-through exposure, nanoparticle suspension in water

Fathead minnow

Acute:
96-h
Endpoints: growth (EC50) , survival (LC50)



Early life stage:
28 days
Endpoints: % hatch, growth, % deformities (EC50, NOEC, LOEC) , survival (LC50)

Xenopus laevis

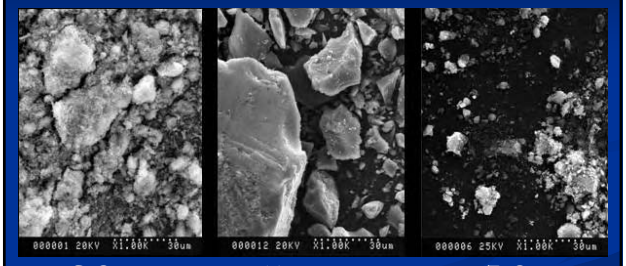
Acute:
96-hour
Endpoints: urvival (LC50)

Chronic:
70 days
Endpoints: ons, metamorph LOEC)

Particles Tested

Oxide	Source	Size (nm)
Copper	CU	>100
	AA	20-40
Iron	CU	>100
	AA	20-40
Zinc	CU	>100
	AA	20-40
Titanium	CU	>100
	AA	32

Particles Synthesized for this Research



CuO TiO_2 ZnO

Sizes required us to use commercial sources of nano-materials

Methods and Materials

- *Xenopus laevis* SEM Preparation
  - Rinsed 3x with Sorenson's phosphate buffer
  - Chemical dehydration Six EtOH exchanges: 10%-100%, ~20min. each
  - Critical point drying with Balzars CP 030 unit to replaces ethanol with CO_2
  - Mount on SEM stub with double sided conductive tape
  - Sputter Coat with Hummer V unit to deposit ~10nm of gold-palladium alloy

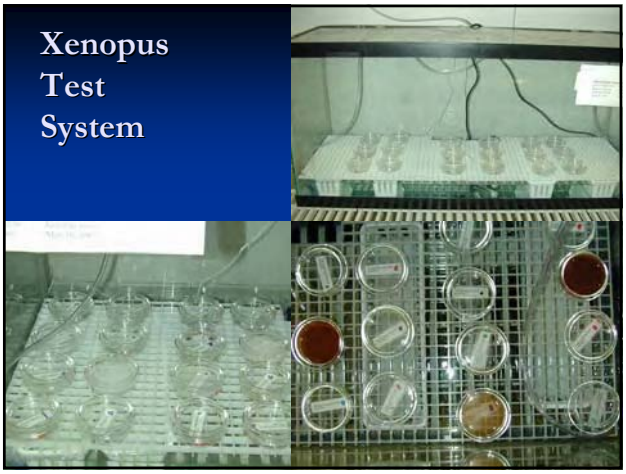
Method for Range Finding Test

- FETAX assay
  - Follow ASTM E1439-98
  - 2 replicates of 8 concentrations including a control (total exposures = 16)
    - 1000, 100, 10, 1, 0.1, 0.01, and 0.001mg/L
    - Control: FETAX solution
      - FETAX solution: NaCl, $NaHCO_3$, $CaCl_2$, $CaSO4{\cdot}2H_2O$, $MgSO_4$, and deionized or distilled water.
  - 5 embryos per exposure

Method for Definitive Test

- FETAX assay
  - Follow ASTM E1439-98
  - 3 replicates of 5 concentrations including a control (total exposures = 18)
    - 1000, 100, 10, 1, and 0.1
    - Control: FETAX solution
      - FETAX solution: NaCl, $NaHCO_3$, $CaCl_2$, $CaSO4{\cdot}2H_2O$, $MgSO_4$, and deionized or distilled water.
  - 10 embryos per exposure

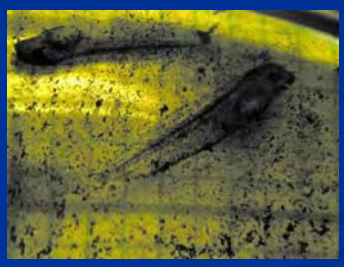
Xenopus Test System



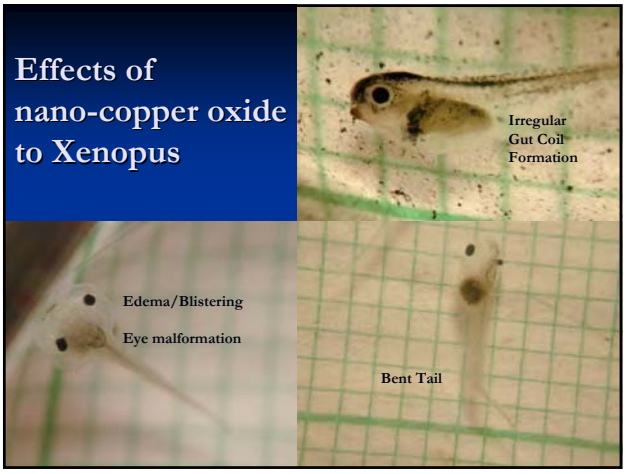
Behavior of nano-iron oxide in water

Uptake of nano-iron oxide to Xenopus

Behavior of nano-copper oxide in water

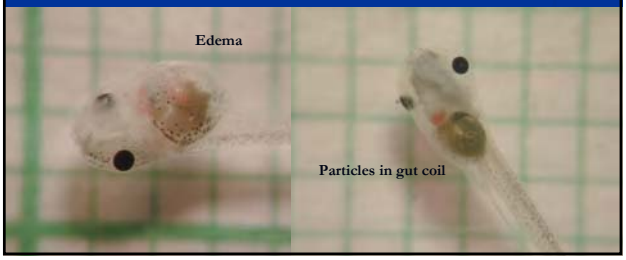


Effects of nano-copper oxide to Xenopus

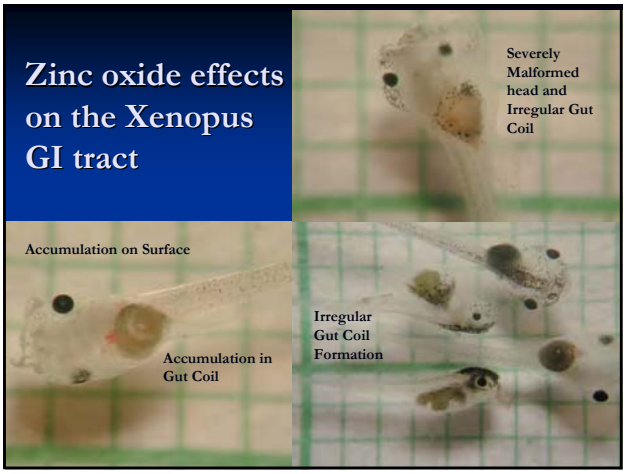


Zinc Oxide behavior in Water

Edema produced by Zinc Oxide



Zinc oxide effects on the Xenopus GI tract



Effects of Zinc Oxide on Xenopus spinal column

Titanium dioxide effect on the Xenopus gastrointestinal tract

Effects of Metal Oxides on Xenopus Development

Range Finding

Compounds	Source	Size	Malformation EC50 (mg/L)
CuO	Clemson		>1000
	Alfa Aesar	23-37	>1000
Fe2O3	Alfa Aesar	20-40nm	>1000
	Alfa Aesar	20-40nm	>1000
TiO2	Clemson		>1000
	Alfa Aesar	32nm	>1000
ZnO	Clemson		16
	Alfa Aesar	40-100nm	65

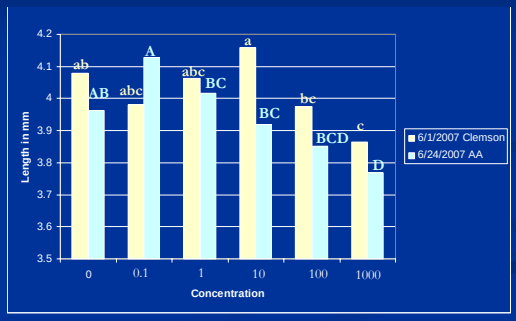
Definitive

Compounds	Source	Size	Malformation EC50 (mg/L)
CuO	Clemson		>1000
	Alfa Aesar	23-37	>1000
ZnO	Clemson		>1000
	Alfa Aesar	40-100nm	31

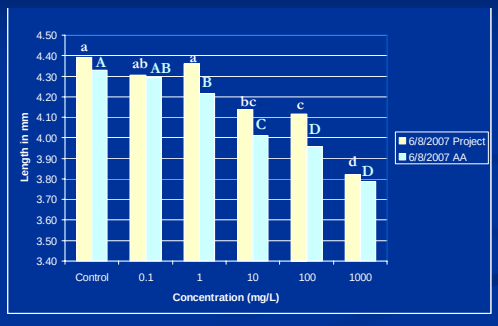
0.8 mg/L for Copper Sulfate

2.8 mg/L for Zinc Sulfate

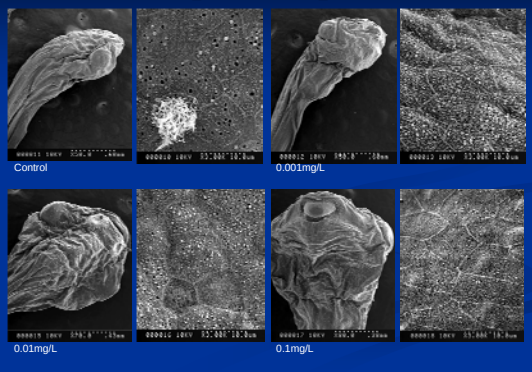
SVL Comparison for ZnO Exposures During FETAX Assays



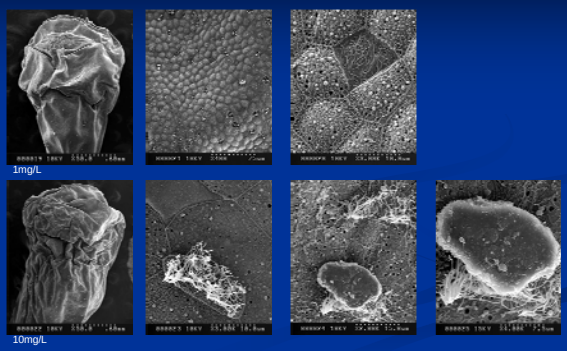
SVL Comparison for CuO FETAX Assay



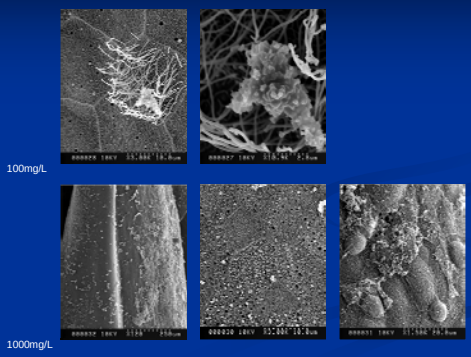
SEM of Tadpoles Exposed to ZnO



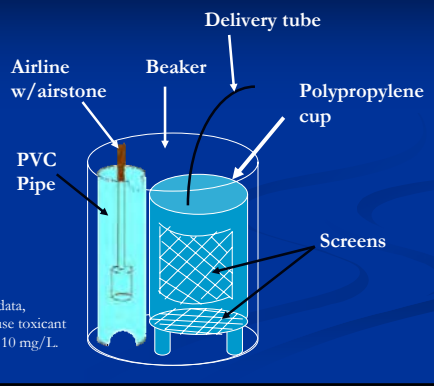
SEM of Tadpoles Exposed to ZnO



SEM of Tadpoles Exposed to ZnO



Chronic Toxicity Exposure Apparatus



Based on chronic data,
We will probably use toxicant
Concentrations of 10 mg/L.

Acknowledgements

- USEPA Grant Number RD-83284201-0
- Texas Tech University Imaging Center
- Dr. EE Smith, Dr. M Grimson

Progress to date

- Funding in place 1 July 2006
- Assessed acute toxicity for Xenopus
- Established zebrafish breeding colonies
- Nanoparticles synthesized and commercial nanoparticles obtained
- Range-finding tests in preparation
  - Chronic tests for Xenopus
  - Acute tests for zebrafish

Mechanistic Dosimetry Models of Nanomaterial Deposition in the Respiratory Tract

Bahman Asgharian, Brian A. Wong, O.T. Price, David Nash, Earl Tewkbury

Division of Computational Biology
The Hamner Institutes for Health Sciences
Research Triangle Park, NC

Study Objectives

1. Deposition measurements of nanosized particle in casts of human and rat nasal URT airways
2. Semi-empirical relationships to predict nanomaterial deposition in the URT airways
3. Respiratory tract deposition models of nanoparticles and nanotubes in humans and rats
4. Measurements of regional and lobar deposition of nanomaterial in the heads and lungs of rats
5. A user-friendly software package to implement models and provide rapid simulation capability

1. Deposition measurements of nanosized particle in casts of human and rat nasal URT airways

Nasal Replicas and Models:

- Human nasal replicas:
  - MRI scans of human nasal passages
  - Input scans into computer to create wire mesh
  - Use stereolithography to create plastic replicas

1. Deposition measurements of nanosized particle in casts of human and rat nasal URT airways

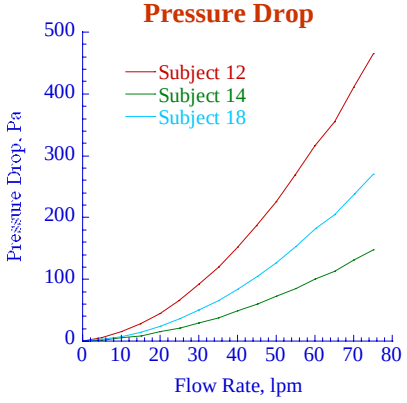
- Rat nasal mold:
  - Low melting point alloy to fill air spaces in cadaver
  - Cast mold in plastic

Deposition of Nanoparticles in Nasal Replicas

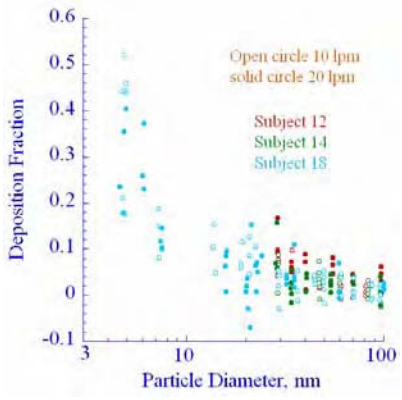
- Generate monodisperse nanoparticles
  - Electrospray generator: 5-30 nm
  - Nebulizer and classifier: 30-100 nm
- Measure particle counts and size at inlet and outlet to nasal mold using Scanning Mobility Particle Sizer (SMPS)
- Calculate Deposition efficiency

$$\eta = 1 - \frac{C_{exit}}{C_{inlet}}$$

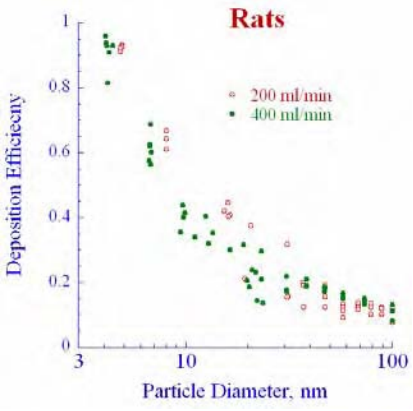
Pressure Drop



Humans



Rats



2. Semi-empirical relationships to predict nanomaterial deposition in the URT airways

Convective diffusion equation:

$$2Sc \cdot u_r^* \frac{\partial c^*}{\partial r^*} + u_z^* \frac{\partial c^*}{\partial \Delta} = 4 \frac{1}{r^*} \frac{\partial}{\partial r^*} \left(r^* \frac{\partial c^*}{\partial r^*} \right) + \frac{1}{Pe^2} \frac{\partial^2 c^*}{\partial \Delta^2}$$

$$Sc = \frac{\nu}{D}$$

$$Pe = \frac{U_0 D_h}{D} \qquad D_h = \frac{4 \times \text{Volume}}{\text{Surface Area}}$$

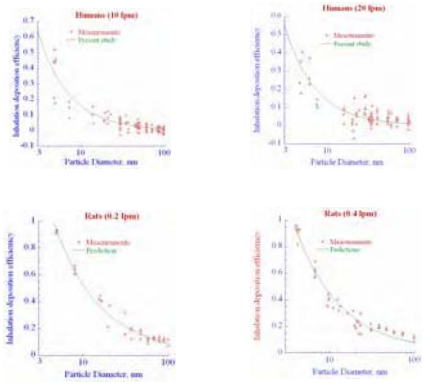
$$\Delta = \frac{D \cdot L_h}{U_0 D_h^2} \qquad L_h = \frac{(\text{Surface Area})^2}{4 \times \pi \times \text{Volume}}$$

$$\Rightarrow \quad \eta = f(\Delta, Sc)$$

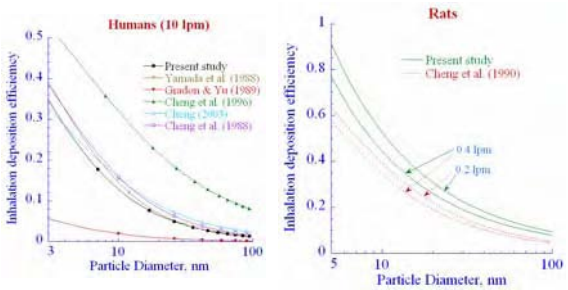
Semi-empirical Relationships

Functional Relationship	Species	Coefficients a	b	c	Correlation coefficient (r^2)
$\eta = a \times Sc^b \Delta^c$	Human	5.005	-0.5126	0.06998	0.76
	Rat	3.896	-0.1582	0.2438	0.76
$\eta = a \times Q^b D^c$	Human	24.61	-0.2975	0.58	0.97
	Rat	7.351	-0.2438	0.402	0.97

Comparison of Fitted curves with data



Model Comparison



3. Respiratory tract deposition models of nanoparticles and nanotubes in humans and rats

I. Extrathoracic airway deposition
- From measurements: semi-empirical models

II. Lung geometry
- Symmetric: Yeh et al. (1979)
- Asymmetric: Koblinger & Hofmann (1990)

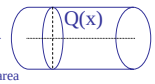
III. Airway flow architecture within the lung
- Uniform lung expansion and contraction
- Uniform velocity equal to average parabolic velocity

IV. Mathematical formulation to calculate deposition in the lung during a breathing cycle
- Deposition by diffusion

III. Lung Ventilation

Uniform Airway expansion:

Continuity Equation: $m_{t+\delta t} - m_t = \dot{m}_{in}\delta t - \dot{m}_{out}\delta t$



$$\frac{\partial A}{\partial t} = -\frac{\partial Q}{\partial x}$$

A: Cross-sectional area

$$Q(x) = Q_0 \frac{DV(x)}{TLV}$$

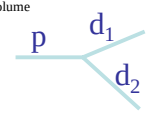
DV: Distal volume
TLV: Total Lung volume

Calculate flow splitting at bifurcations

$$Q_{p|_{--}} = \left(1 - \frac{V_p}{DV_p}\right) Q_{p|_{--}}$$ V_p: Airway volume

$$Q_{d_1|_{--}} = \frac{DV_{d_1}}{DV_p - V_p} Q_{p|_{--}}$$

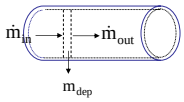
$$Q_{d_2|_{--}} = \frac{DV_{d_2}}{DV_p - V_p} Q_{p|_{--}}$$



IV. Mathematical Model for Particle deposition

Particle mass balance per airway:

$$m_{t+\delta t} - m_t = \dot{m}_{in}\delta t - \dot{m}_{out}\delta t - m_{dep}$$

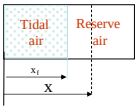


$$\frac{\partial(AC)}{\partial t} + \frac{\partial(QC)}{\partial x} = \frac{\partial}{\partial x}\left(DA\frac{\partial C}{\partial x}\right) - \lambda_d C$$

λ_d: mass lost per unit time per unit volume

Solution:

$$C(x,t) = C_0 e^{-(\lambda_r + \lambda_d)(t - t_0)} erfc\left(\frac{\xi}{2\sqrt{D(t-t_0)}}\right)$$

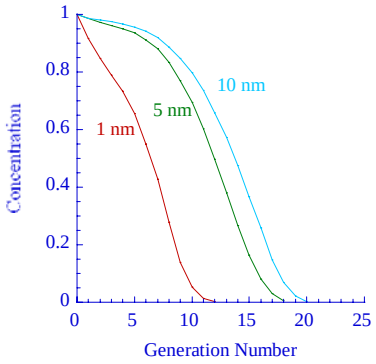


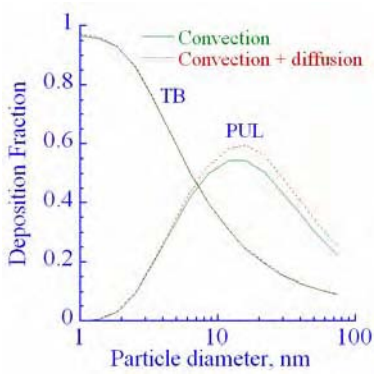
Calculation Steps:

1. Calculate deposition efficiencies per airway.
2. Particle concentrations at the inlet and exit of all airways.
3. Airflow rates at the inlet and exit of airways.
4. The time it takes for the aerosol front to pass the inlet and exit of each airway.
5. Calculate Losses per airway:

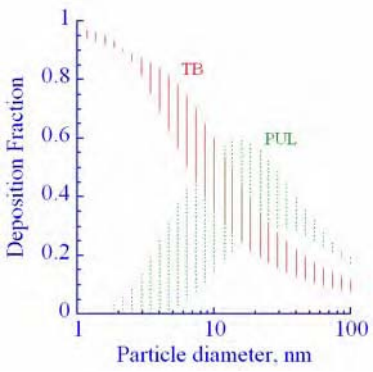
$$\text{Losses} = \int_0^{\text{time}} \int_0^{\text{Distance}} \lambda_d C A dx dt$$

Concentration at the end of inhalation

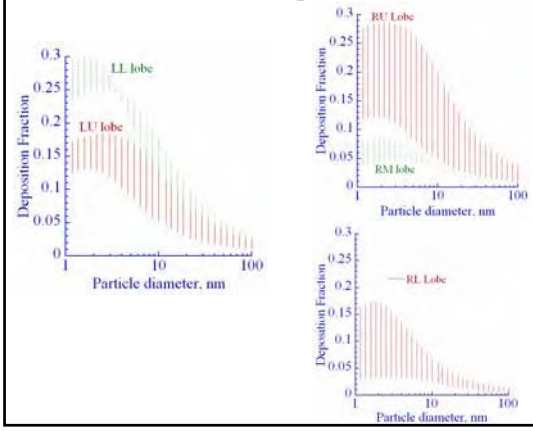




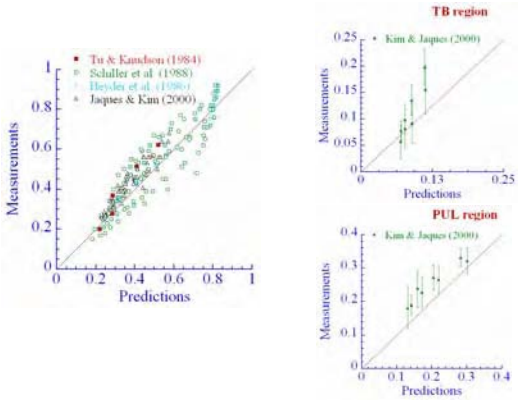
Regional deposition



Lobar Deposition



Model Verification

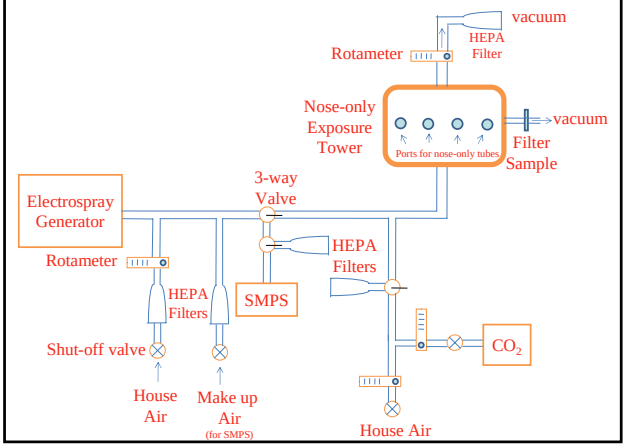


4. Measurements of regional and lobar deposition of nanomaterial in the heads and lungs of rats

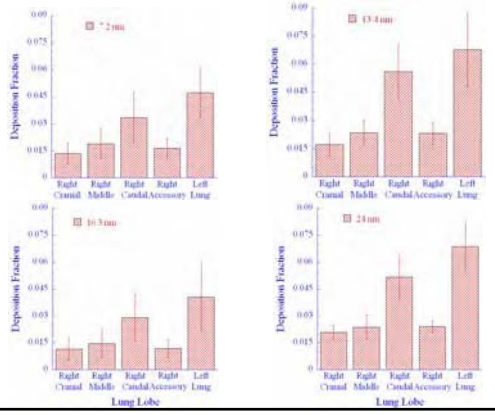
- Generation system: TSI Electrospray aerosol generator
  - Particles: $^{59}FeCl_3$ (73 mCi/mg of Fe, 44 days half life)
  - Particle size: 5 nm to 100 nm
- Exposure system: Cannon nose-only tower
  - Animals: Long-Evans rats
  - Exposure duration: 30 minutes
- Detection system: SMPS for size measurements
  Gamma counter for activity (mass)

Experimental procedure

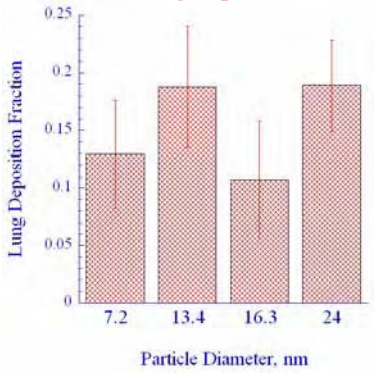
- ✓ Prior to exposure (10 minutes)
  - Clean air through the nose-only tower
  - Baseline measurement of breathing rates for each animal (Buxco system)
  - Particle size distribution measurements using SMPS
- ✓ During exposure (30 minutes)
  - Breathing rate measurements
  - Filter sample collected as a port of the nose-only tower
- ✓ Post exposure
  - Particle size distribution measurement
  - Animals asphyxiated by a direct flow of CO_2 into the nose-only tower
  - Tissue samples collected in a gamma counter and activities measured



Lobar Deposition



Lung Deposition



Summary

- Deposition fractions in the nasal airways of humans and rats were measured for particles sizes between 5 nm to 100 nm
- A semi-empirical deposition efficiency formula was obtained as a function of Sc and Δ.
- Model of particle deposition in the lung was extended to ultrafine (nano) size range by including axial diffusion and convective mixing (dispersion)
- Lobar and regional deposition of nanoparticles were measured in Long-Evans rats

Preparation and Application of Stabilized Fe-Pd Nanoparticles for *in situ* Dechlorination in Soils and Groundwater: Factors Affecting Particle Transport and Reactivity

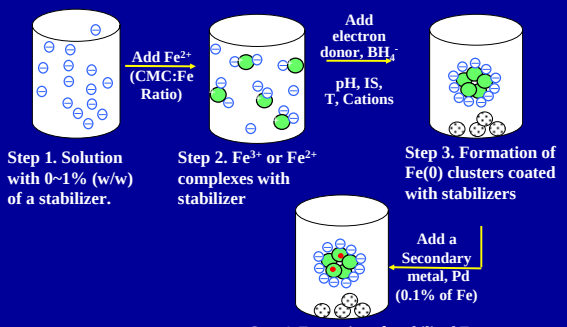
Progress Report II: September 5, 2007

Don Zhao, Chris Roberts[1], F. He and J.C. Liu[1]
Department of Civil Engineering
[1] Department of Chemical Engineering
Auburn University, Auburn, AL 36849

Primary Accomplishments in Year 2

- Prepared nanoparticles of various size using CMC (carboxymethyl cellulose) as a stabilizer
- Tested effects of particle stabilization on reactivity
- Tested transport behaviors of ZVI nanoparticles in porous media
- Tested degradation of TCE in soils
- Pilot tested in situ dechlorination in soils using stabilized ZVI nanoparticles

Size-Controlled Synthesis of ZVI Nanoparticles Using CMC as a Stabilizer



Step 1. Solution with 0~1% (w/w) of a stabilizer.

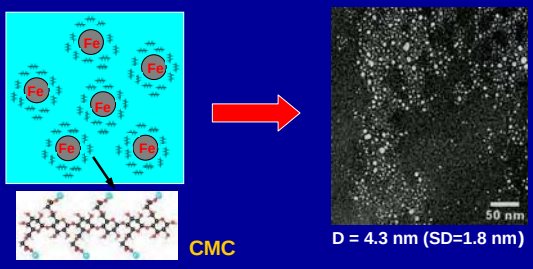
Step 2. Fe^{3+} or Fe^{2+} complexes with stabilizer

Step 3. Formation of Fe(0) clusters coated with stabilizers

Step 4. Formation of stabilized Fe-Pd bimetallic nanoparticles.

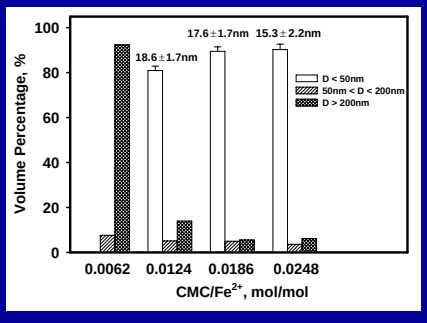
He et al., I&EC Res. 2007, 46(1), 29-34.

ZVI nanoparticles stabilized with a CMC (90k M.W.)



D = 4.3 nm (SD=1.8 nm)

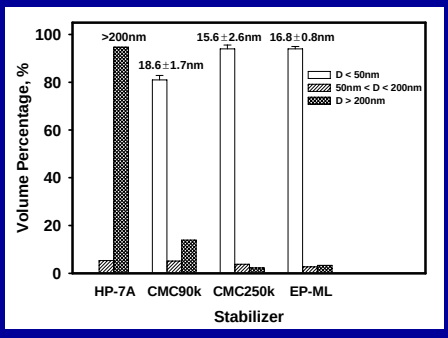
Size distribution of ZVI nanoparticles synthesized at various CMC-to-Fe molar ratios



Fe^{2+} = 0.1 g/L; CMC M.W. = 90k, temp = 22° C

He and Zhao, Environ. Sci. Technol. 2007, 41, 6216-6221.

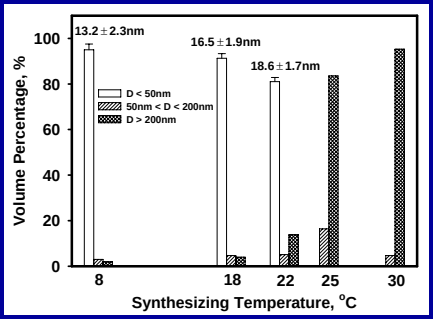
Size distribution of ZVI nanoparticles synthesized with CMC of Various M.W.



Fe^{2+} = 0.1 g/L; CMC = 0.2% w/w, temp = 22° C

He and Zhao, Environ. Sci. Technol. 2007, 41, 6216-6221.

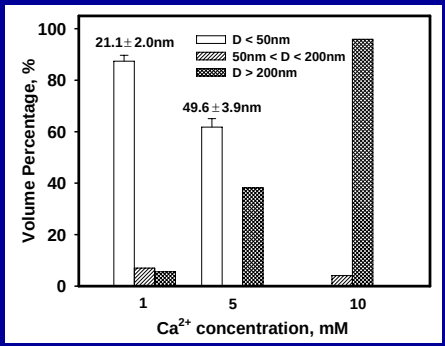
Effect of Temperature on ZVI Particle Size



Fe^{2+} = 0.1 g/L; CMC90k = 0.2% w/w

He and Zhao, Environ. Sci. Technol. 2007, 41, 6216-6221.

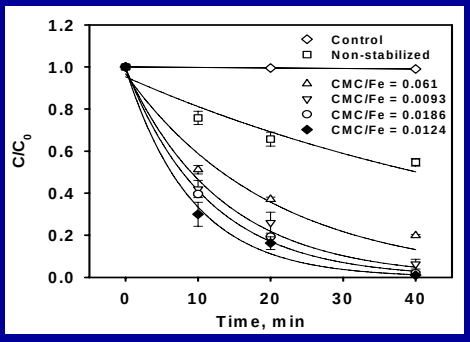
Effect of Calcium on ZVI Particle Size



Fe^{2+} = 0.1 g/L; CMC90k = 0.2% w/w

He and Zhao, Environ. Sci. Technol. 2007, 41, 6216-6221.

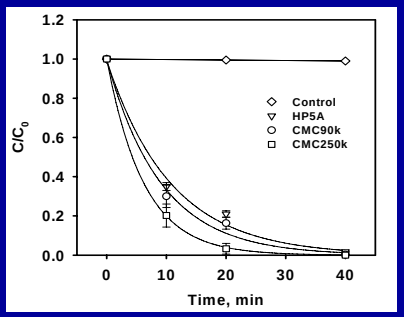
Effect of CMC:Fe Molar Ratio on TCE Degradation



TCE (C_0) = 20 mg/L, Fe = 0.1 g/L, Pd:Fe = 1.0 mg Pd/g Fe, pH = 8.3.

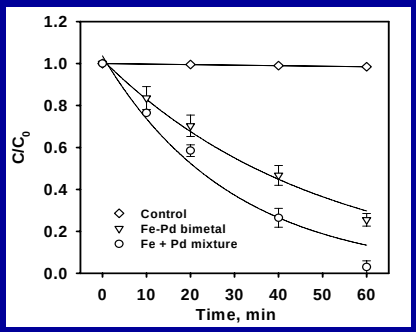
He and Zhao, Applied Catalysis B. 2007 (under review)

Effect of CMC Type on TCE Degradation



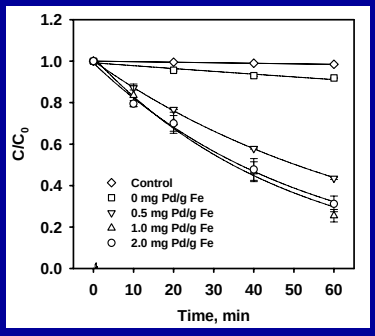
TCE (C_0) = 20 mg/L, Fe = 0.1 g/L, CMC=0.2%, Pd:Fe = 1.0 mg Pd/g Fe, pH = 8.3. *He and Zhao, Applied Catalysis B. 2007 (under review)*

Effect of Pd Nanoparticles on TCE Degradation



TCE (C_0) = 20 mg/L, Fe = 0.1 g/L, CMC90=0.2%, Pd:Fe = 1.0 mg Pd/g Fe, pH = 8.3. *He and Zhao, Applied Catalysis B. 2007 (under review)*

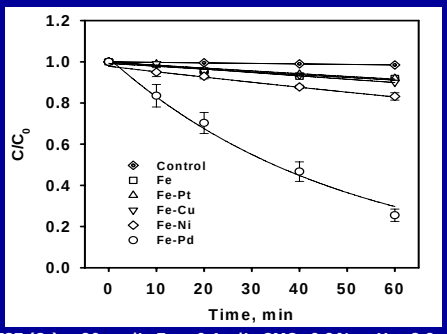
Effect of Pd Loading on TCE Degradation



TCE (C_0) = 20 mg/L, Fe = 0.1 g/L, CMC=0.2%, pH = 8.3.

He and Zhao, Applied Catalysis B. 2007 (under review)

Effect of Various Metal Catalysts on TCE Degradation



TCE (C_0) = 20 mg/L, Fe = 0.1 g/L, CMC=0.2%, pH = 8.3.

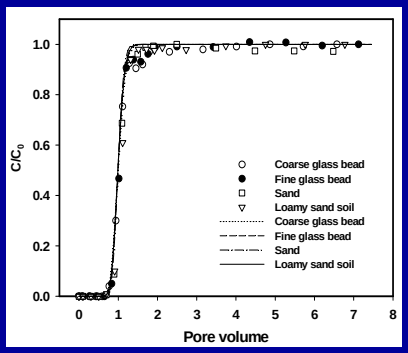
He and Zhao, Applied Catalysis B. 2007 (under review)

Transport of Stabilized ZVI Nanoparticles Media Properties

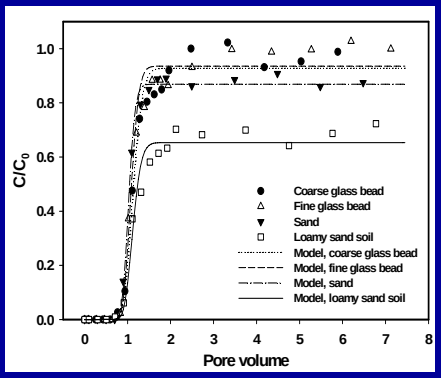
Porous media	dc mm	bulk density g/mL	porosity	pore velocity cm/s	dispersivity cm^2/h	r^2
Coarse glass bead	1.1	1.49	0.421	0.0302	26.2	0.993
Fine glass bead	0.52	1.57	0.388	0.0327	21.3	0.993
Sand	0.33	1.73	0.360	0.0353	18.1	0.990
Loamy sand soil	0.33	1.74	0.355	0.0358	25.9	0.978

Column Test Setup

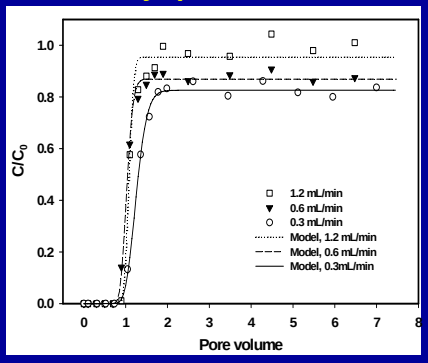
Breakthrough Curves of Bromide in Porous Media



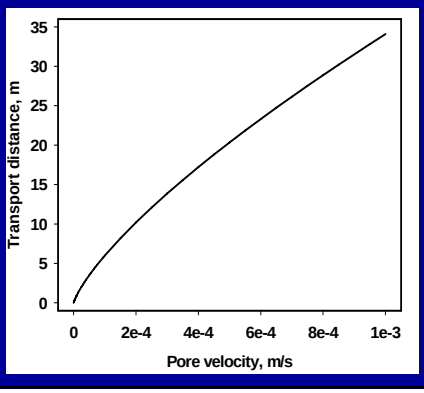
Breakthrough Curves of ZVI in Porous Media



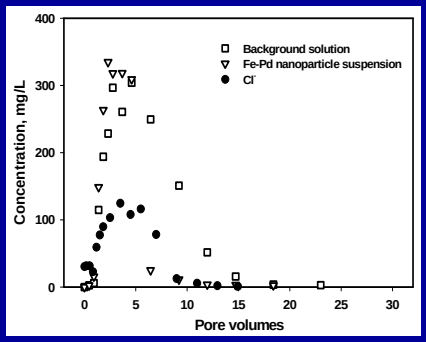
Breakthrough Curves of ZVI through a Sand at Various Pore velocity: (0.017, 0.035, 0.071 cm/s)



Distance over which 99% of CMC-stabilized nanoparticles are removed in the sand at different pore velocities



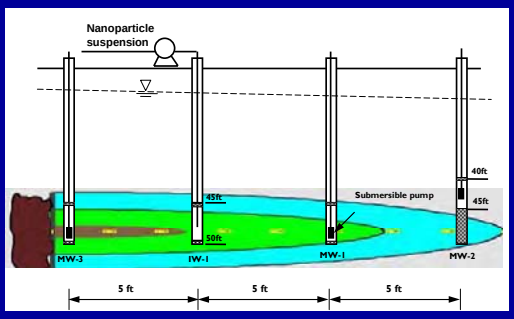
Degradation of TCE in a Sand Column and Production of Chloride



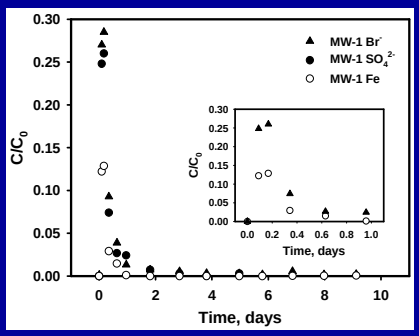
TCE = 14.6 mg, Fe = 0.5 g/L, Pd/Fe = 0.1 wt%, CMC = 0.4 wt%, pore velocity = 0.0118 cm/s, EBCT = 84 min

Pilot-Tests at an Alabama Site

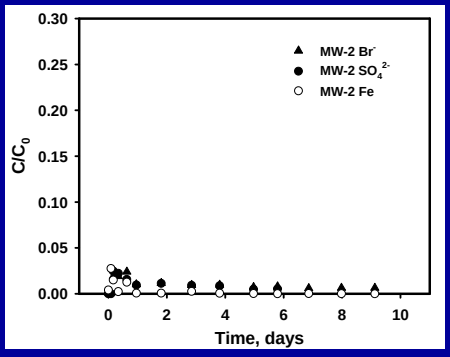
Pilot-Tests at an Alabama Site



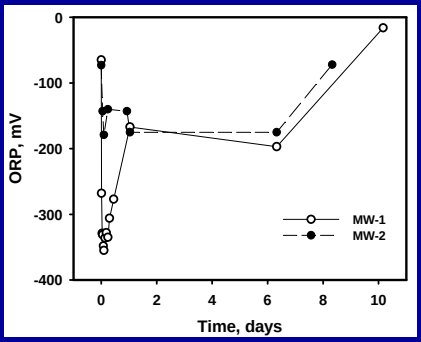
Concentration Histories of Tracers and ZVI in MW 1



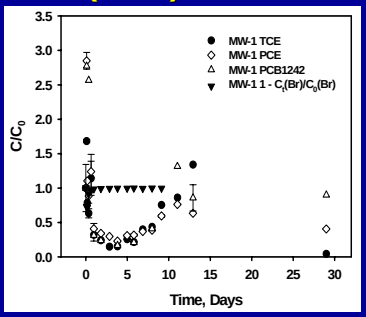
Concentration Histories of Tracers and ZVI in MW 2



Change in ORP in the Two MW's

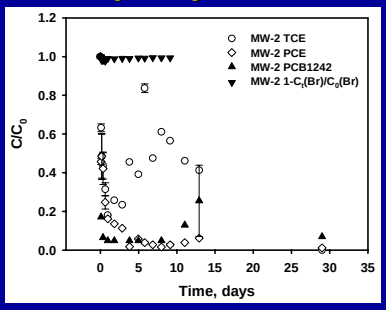


Concentration Histories of TCE, PCE and PCB-(1242) in MW 1



C_0 in MW 1: PCE=1225 ppb, TCE=1655 ppb, PCB1242=21

Concentration Histories of TCE, PCE and PCB-(1242) in MW 2



C_0 in MW 2: PCE=4133 ppb, TCE=3710 ppb, PCB1242=62.

Summary

- Developed a method for synthesizing ZVI nanoparticles of controllable size and soil mobility and reactivity
- Factors such as CMC M.W., CMC/Fe ratio, pH, and T can greatly affect transport and reactivity of nanoparticles
- The stabilized ZVI nanoparticles can be delivered and distributed in soils
- The nanoparticles can effectively degrade NAPLs in soils and groundwater, and may boost biodegradation

Publications

- 8 journal papers published
- 5 more under review
- 1 U.S. patent
- ~20 presentations
- 2 Pilot tests

Acknowledgements

- USEPA STAR Grant (GR832373)
- Geomatrix Consultant, Oakland, CA
- Golder Associates, Atlanta, GA
- Dr. Gupta in Chemical Engineering Department for DLS analysis

Responses of Lung Cells to Metals in Manufactured Nanoparticles

EPA Project 2004-STAR-1-A1
Grant Number RD 8317230

Co-Investigators / John M. Veranth, Chris A. Reilly, Gary S. Yost
Faculty Collaborators
N. Shane Cutler, Philip Moos, Agnes Ostafin
Students & Staff
Cassandra Deering, Mike Koch, Erin Kaser, Diane Lanza
Department of Pharmacology & Toxicology
University of Utah

Interagency Workshop on the Environmental Implications of Nanotechnology
Washington, DC
September 5-7, 2007

Grant # RD 83172301

Where we are - Where we are going

- Paper comparing lung epithelial cell responses to micron- and nanosized oxide powder pairs published in Particle & Fibre Toxicology (2007)
- Continuing evidence that nano-sized metal oxides have moderate potency to epithelial cells when compared to other environmental and occupational agonists.
- Studying other cell types in lung - vascular endothelial cells appear to be sensitive to particles.
- Studying activation of cell signaling pathways and changes in gene transcription.
- Ongoing testing for artifacts that may confound results.

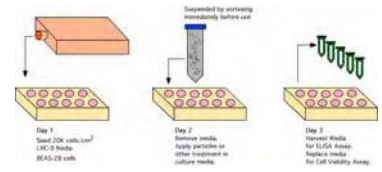
Project Overview

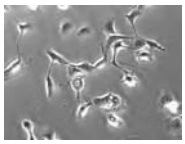
- **Hypothesis:** Transition metals in particles induce pro-inflammatory cytokine production via reactive oxygen species production.
  - **Assumption:** Due to their high surface area nanoparticles are like to induce larger responses in cells than their micron-sized counterparts.
- Approach:
  - Commercially available particles of metal oxides.
  - Physical characterization of particles.
  - *In vitro* cell culture screening assays & *in vivo* confirmation.
  - Followup studies based on new evidence.

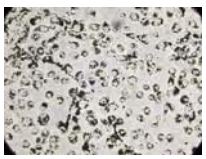
Particle Types

- SiO_2
  - Thermally generated nanoparticles
  - Fluorescent (aqueous process) nanoparticles
  - Lab synthesized & surface modified nanoparticles
  - Comparison to micron-sized: amorphous and Min-U-Sil
- Other Oxides
  - Supermicron- and nano-sized. Manufactured powders.
  - TiO_2, Fe_2O_3, Al_2O_3, NiO, CeO_2, Zn0
- Comparisons to environmental PM
  - Soil-derived dust
  - Diesel PM

In vitro Particle Toxicology



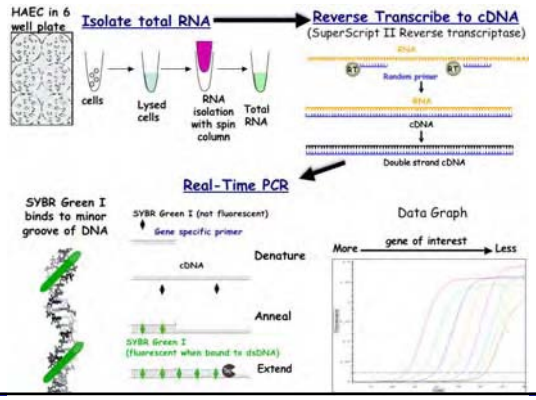




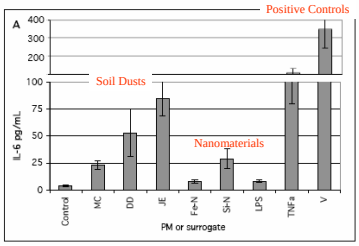
Cytokines
Small peptide extracellular signaling molecules that are important for regulating cell growth, tissue differentiation, inflammation, and other processes. Interleukin-6 (IL-6) is a marker of inflammation. IL-6 is increased in humans exposed to high levels of air pollution and in persons with lung disease.

ELISA
Enzyme-linked immunosorbent assay. Widely used for measurement of trace proteins in biological media.

Quantitative Real Time PCR for changes in gene expression

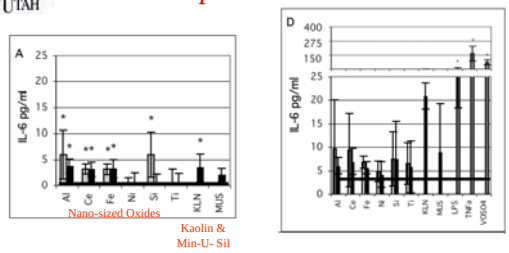


In Lung Epithelial Cells the Oxide Nanoparticles Have Low Potency Compared to Soil-Derived Dusts and Vanadium



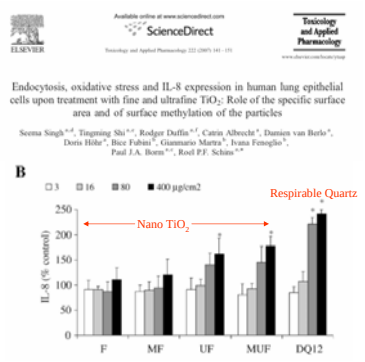
- BEAS-2B immortalized lung epithelial cells in KGM media.
- Soil Dusts
  - PM2.5-enriched material representative of unpaved roads at three sites.
- Positive Controls:
  - TNF-α, a macrophage-generated cytokine known to induce IL-6.
  - V (Soluble vanadium), the potent component of residual oil fly ash.

Two Perspectives on the Same Data



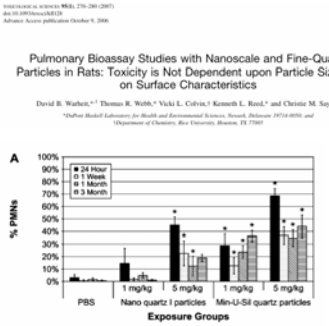
- Panel A shows a single experiment that found statistically significant responses to nanoparticles
- Panel B includes additional biological replicates and compares results to positive controls. Note break in Y-axis scale.

Other studies have reported similar results.



- Fine (40-300 nm TiO_2, Ultrafine (20-80 nm) TiO_2, as-received and methylated.
- Cytokine response of A549 cells.
- DQ12 respirable quartz was more potent than methylated nanoparticles of TiO_2.
- Significant responses only at **very high doses** - More on dosimetry later.

Other studies have reported similar results.



- Animal exposure.
- Endpoint - neutrophils in lung, a marker of acute inflammation.
- Min-U-Sil, a mechanically produced crystalline silica containing particles up to 2 µm was more potent than 30-65 nm nanoquartz particles.

So, are metal oxide nanoparticles "safe"?

Evidence to Date (Epithelial cells, cytokine assays):

- Manufactured metal oxide nanoparticles may pose a risk comparable to other ambient and occupational particle types such as micron-sized crystalline silica.

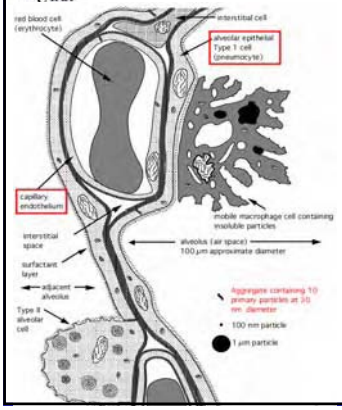
But work is ongoing:

- Other biological endpoints.
- Other cell types in the lung.
- Cellular mechanisms linking particles to systemic responses.

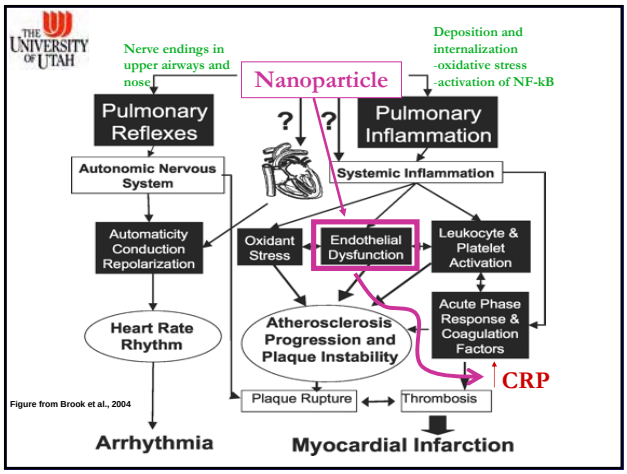
Goals

- Generalizing from cell culture to whole animal and human responses requires understanding biochemical mechanisms.
- Elucidating particle surface characteristics relevant for biological responses.

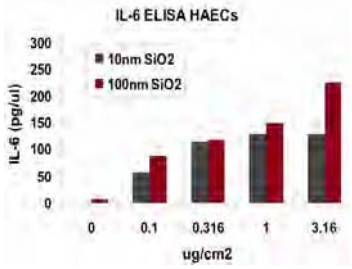
Multiple Cell Types in the Lung



- Vascular endothelial cells are in close proximity to the airspace.
- Translocation of nanoparticles from the lung is well documented.
- Cardiovascular effects are reported in response to environmental air pollution.
- Current Work: Investigating responses of endothelial cells *in vitro*.

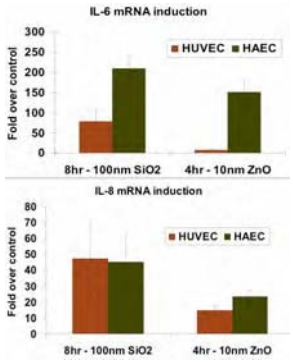


Evidence that some nanosilica types induce proinflammatory signaling in endothelial cells.



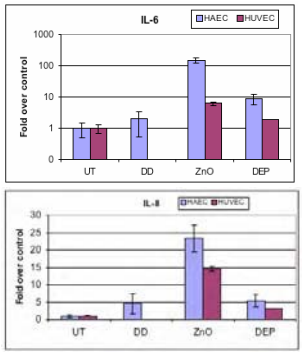
- Human aortic endothelial cells show statistically significant IL-6 ┼ response at much lower concentrations than BEAS-2B epithelial cells.
- 100 nm silica appears more potent than 10 nm amorphous silica.
- Followup work will focus on surface-modified SiO2 particles. (amine and carboxyl).

Two endothelial cell types Two nanoparticle types



- Quantitative PCR for Changes in Regulation of Inflammation-Related Genes
- Both umbilical vein and aortic endothelial cells are responsive.
- Nano-zinc is comparable in potency to the silica.
- Work in progress.

Comparisons to other particle types.



- Treatments
  - UT = untreated
  - DD = desert dust PM2.5
  - ZnO = 10 nm
  - DEP = Diesel Emission Particulate
- Endothelial cells are more responsive to nano-Zn than to diesel soot (an incidental nanomaterial).
- Future work on cellular response mechanisms.

Particle Dosimetry Issues

- Important issue with *in vitro* particle studies.
- Responses are often seen only at doses much higher concentration than are plausible for lung from inhalation exposure.
- However, this may reflect the artifacts of cell culture.
  - Lung surfactant is 0.05-0.2 μm thick compared to several mm of cell culture media.
  - Seagrave reports much higher response with cells grown at air-liquid interface.

cells 1micron thick
(not to scale)

- Our doses are within range of similar studies.

Ongoing Work

- Continued comparisons between lung epithelial and endothelial cells *in vitro*.
- Use specific inhibitors to study cell signaling pathways activated by the more potent types of nanoparticles.
- Animal exposure (intratrachael aspiration) to validate *in vitro* results.

A Toxicogenomics Approach for Assessing the Safety of Single-Walled Carbon Nanotubes in Human Skin and Lung Cells

Presenter: Mary Jane Cunningham, Ph.D.,

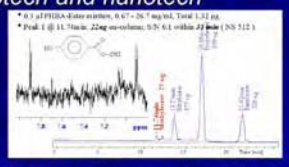
HARC

Toxicogenomics

- Toxicogenomics: using OMICs to assess safety

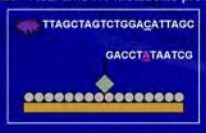
Focus: Merging biotech and nanotech

Genomics=
Gene Expression Microarrays



Metabonomics= Real-time HT Metabolite profiling

Proteomics=
2D Gel Electrophoresis + Mass Spectroscopy



Pharmacogenomics= SNPs profiling

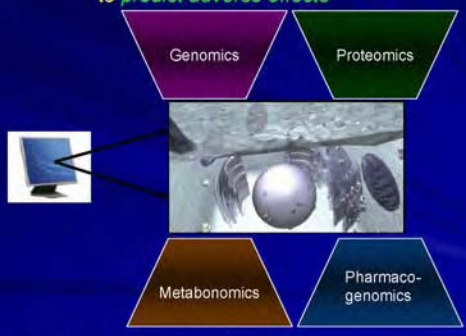
Alternative Method for Predicting Toxicity

Objectives:

- Obtain expression profiles (EP) of:
  - -Known nanomaterials
  - -Unknown nanomaterials
  - -Compare EPs ➡ to ID toxic effects
- Use "systems biology" approach to:
  - -Perturb the biological system
  - -Reiteratively sample over time or dose
  - -"Data-driven" approach +"Reverse engineer" cellular pathways

Alternative Method for Predicting Toxicity

Ultimate Goal: create a virtual cell interaction network to predict adverse effects

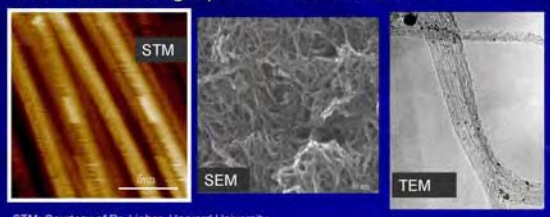


*Virtual reality cell image courtesy of Netera Alliance

Manufacturing and Analysis of Single-Walled Carbon Nanotubes (SWNT)

SWNT: Manufacture and Characterization

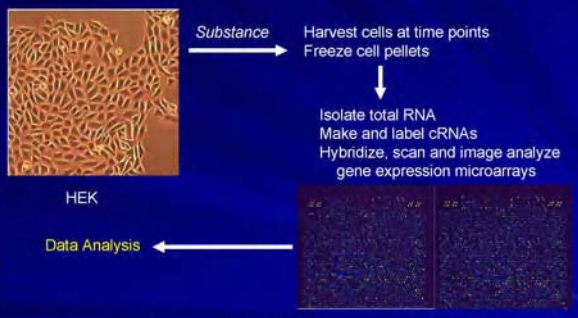
- Manufactured by a modified chemical vapor deposition method (OU, SWeNT)
  - -Less than 1% heavy metal contamination
  - -Two predominant species: (6,5) and (7,5)
  - -Avg diameter=2.76nm
- Electron micrographs: SEM, TEM, STM



STM: Courtesy of Dr. Lieber, Harvard University

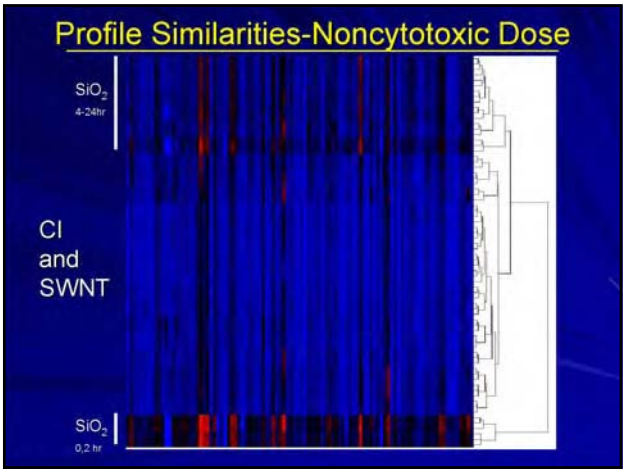
Previous Results with Primary Human Epidermal Keratinocytes (Dermal Exposure Route)

Experimental Design

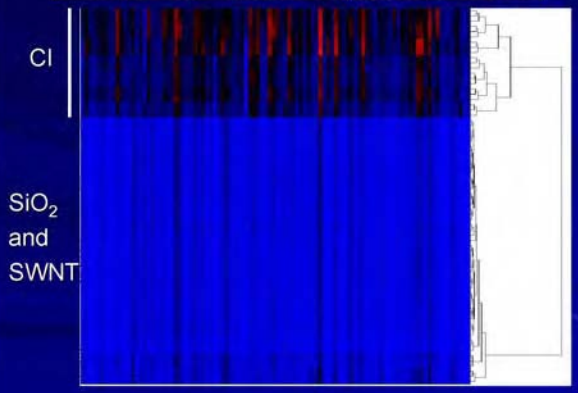


Microarrays: Screen for 10,000s of gene activities simultaneously; mfg by GE Healthcare, one-color, 30 bp oligos embedded in gel matrix

Profile Similarities-Noncytotoxic Dose



Profile Similarities-Cytotoxic Dose



Summary of Results with Skin Cells

- Noncytotoxic dose:
  - -EP of SWNT is more similar to EP of CI (nontoxic control)
  - -EP of SiO_2 is the most active
- Cytotoxic dose:
  - -EP of SWNT is more similar to EP of SiO_2 (toxic control)
  - -EP of CI is most active
- Significantly-expressed genes with SiO_2 correlated with previous literature
  - -genes involved in membrane restoration/remodeling, inflammation and irritation responses

Postdoctoral Fellow: Dr. Mrinal Shah

Studies with Primary Human Bronchial Epithelial Cells (Inhalation Exposure Route)

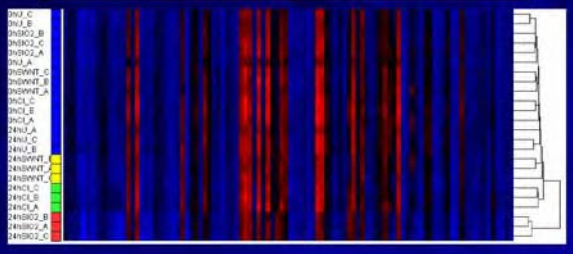
Phase Contrast Photomicrographs

Human Epidermal Keratinocytes

Human Bronchial Epithelial Cells

- HEK culture is almost all basal keratinocytes
- HBE culture contains cells of different morphologies
  - *Possible cell types may include:* Pseudostratified columnar cells, non-ciliated cells (Goblet cells), simple ciliated columnar cells, non-ciliated rounded secretory cells (Clara cells)
- Growth of HEK more robust than HBE, could not bank enough cells for full time course, need to reformulate media and modify growth conditions

GEP Results



Observations:

-Profiles from cells exposed to SiO_2 (pos control) differ from profiles of cells exposed to any of the other nanomaterials.

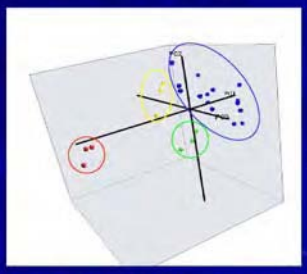
-SWNT profiles are more similar to carbonyl iron (neg control) than to SiO_2.

Parameters: Hierarchical clustering, Euclid distance metric, single linkage.
Red=High expression, Blue=Low expression

Initial Data Analysis

Statistical variability among triplicates	
Sample	CV(%)
0h-SiO_2	6.3
0h-CI	6.6
0h-SWNT	5.9
0h-Untreated	6.2
24h-SiO_2	5.8
24h-CI	8.1
24h-SWNT	6.9
24h-Untreated	6.3

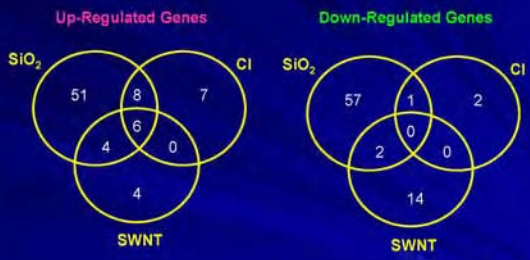
Principal Components Analysis (PCA)



Silica CI SWNT Untreated

PCA from (GeneLinker™ Platinum)

Significantly-Expressed Genes for HBE



Observations:

-Higher number of genes expressed in SiO_2 treatment

-Six genes in common as up-regulated; may be particulate-induced genes

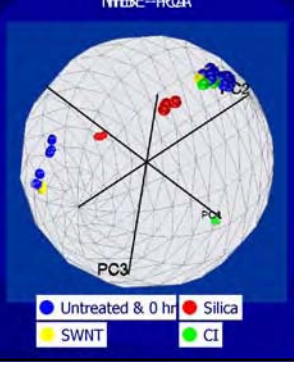
Summary of Results with Lung Cells

- HBE not as robust as HEK, could not perform complete time course
  -obtained EP at 0 and 24 hr.
- As with HEK, very low CVs between array replicates
- EP of SiO_2 is most different, most active
- EP of SWNT is more similar to EP of CI
- Significantly-expressed genes were similar to those found with HEK
  -found genes active in inflammation, irritation and membrane remodeling
- Any adverse effects observed with SWNT seem to be limited to local inflammation caused by the physical presence of particulate material.

Postdoctoral Fellow: Dr. Carolina Lema

Comparison: EPs from Skin and Lung Cells

PCA from GeneLinker™ Platinum



Principal Components Analysis (PCA)

-Maximum difference seen between tissue types

-EP of SWNT in both NHBE and HEK is similar to Untreated samples

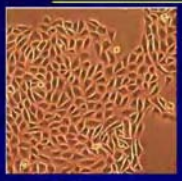
-Observed approx. 10X more overall activity with skin cells

-Very similar types of genes expressed between cell types

Postdoctoral Fellow: Dr. Mrinal Shah

New Directions

Experimental Design

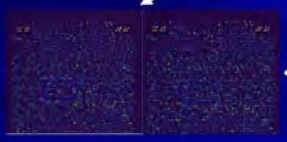


Cells

Substance → Harvest matched cell cultures at time points

mRNA and miRNA:
-Freeze cell pellets
-Isolate total RNA; make and label cRNAs; hybridize, scan, image analyze microarrays

Proteomics:
-Lyse cell cultures
-Run 1DE, 2nd DE
-MALDI TOF/TOF to determine mass and tryptic peptide sequence
(UTMB Galveston)

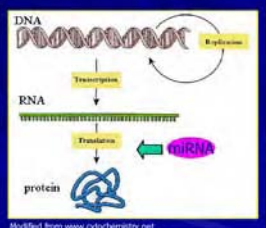


Data Analysis

mRNA: GE Healthcare
miRNA: Agilent Technologies

miRNA Expression Profiling

- 2006 Nobel Prize in Medicine for discoverers
- microRNA or miRNAs:
  - -Small single-stranded RNA molecules (21-23 bp)
  - -Non-coding RNAs that regulate gene expression
  - -Regulate one-third of all human genes
  - -Complementary to one or more mRNAs
  - -***Degrade or repress target mRNA(s)***
  - -Cell proliferation, differentiation; cellular stress
  - -Role in toxicity not yet explored
  - -Possible toxicity biomarkers?



Modified from www.cytochemistry.net

Summary from Proteomics and miRNA

Protein Expression:

- Only 6 proteins significantly expressed at 24hr.
- Need more time points
- Switch to large format gels or LFQMS

miRNA Expression:

- CVs are <20% between array replicates
- Most miRNA expression seen with SiO_2
- 71 miRNAs significantly expressed
- Pathway analysis and interpretation ongoing
  - -no databases for pathways of miRNAs
  - -relationships will need to be done manually with comparison of data mostly from plants and microorganisms

Postdoctoral Fellow: Dr. Carolina Lema

Acknowledgements

HARC's Toxicogenomics Team:

- Carolina Lema, Postdoctoral Fellow
- Mrinal Shah, Postdoctoral Fellow

Collaborators:

- Daniel Resasco (OU), Leandro Balzano (SWeNT)
- Ed Dougherty, Ulisses Braga-Neto, Amin Zollanvari, Yufei Xiao (Texas A&M)
- Scott Magnuson, Michael Falduto (GenUs BioSystems)
- Bo Curry and team (Agilent Technologies)
- John Wiktorowicz and team (UTMB Galveston)

Funding:

- National Science Foundation (BES-0436366 and BES-0536679)
- HARC and the George P. Mitchell family

Various Forms of SWNT

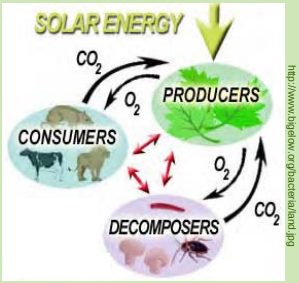
0.35 g Raw 0.45 g Purified

Microbial Impacts of Engineered Nanoparticles

Pedro Alvarez (Delina Lyon*) & Mark Wiesner

Interagency Workshop on the Environmental Implications of Nanotechnology 2007

Why examine effect of nanomaterials on bacteria?



http://www.bigelow.org/bacteria/land.jpg

Implications

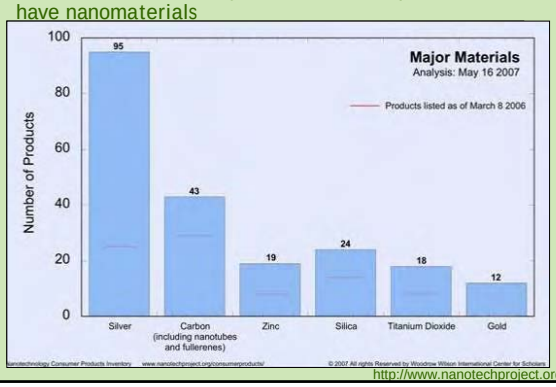
- Disposal/accidental discharge can effect microbial ecology and disrupt biogeochemical cycles
- Antibacterial activity indicative of toxicity to higher level organisms

Applications

- Use for water treatment?
- Other disinfection

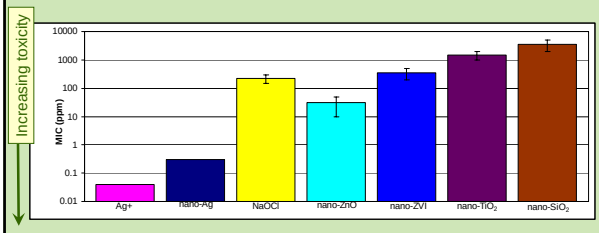
Nano in Consumer Products

Wilson Center inventory: >475 consumer products claim to have nanomaterials

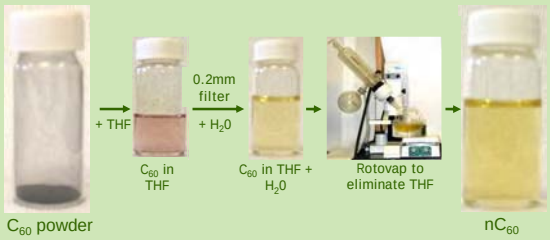


http://www.nanotechproject.org/44

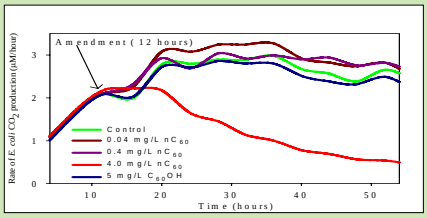
Antibacterial activity of common nanomaterials



Antibacterial activity of environmentally relevant fullerenes

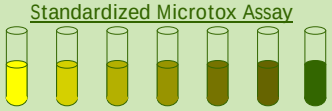


nC_{60} is antibacterial



Respiration in *E. coli* decrease/ceases after exposure to nC_{60}

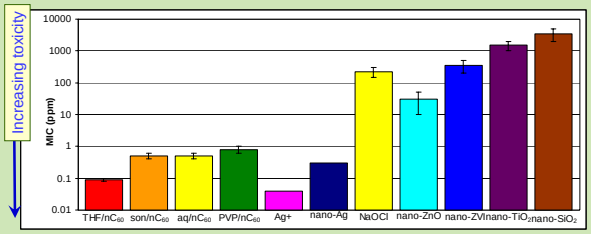
Standardized Microtox Assay



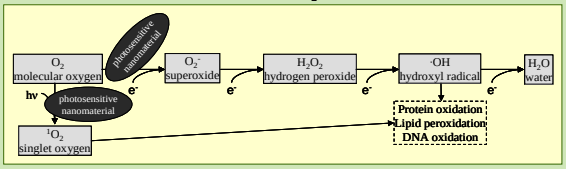
Vibrio fischeri (luminescent bacteria) with increasing concentrations of nC_{60}

Compound	EC_{50} (mg/L)
nC_{60}	1.6
Benzene	2.0
Sodium azide	43-66

nC_{60} is more toxic to bacteria than many other common nanomaterials

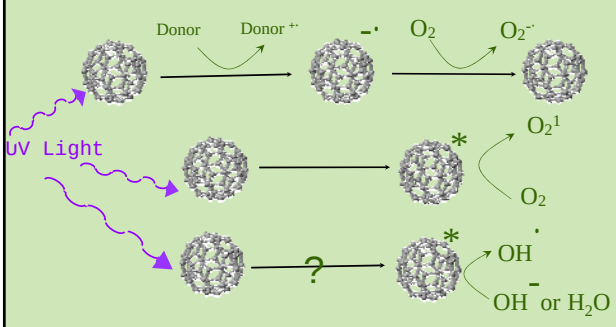


Antibacterial activities have been linked to ROS production

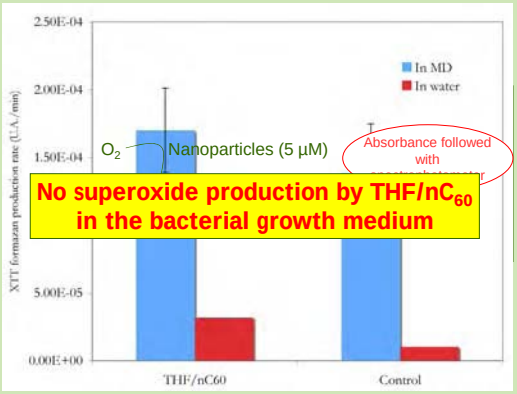


- In some cases, cytotoxicity is linked to photochemical ROS production
  - TiO_2
  - nC_{60}
- nC_{60} is antibacterial in dark, under anaerobic conditions

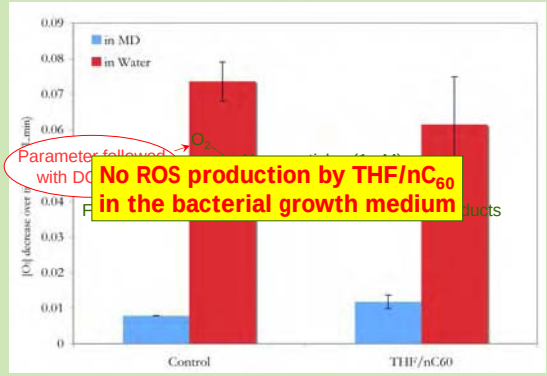
Production of ROS by fullerenes



Detection of superoxide ion ($O_2^{-\cdot}$)



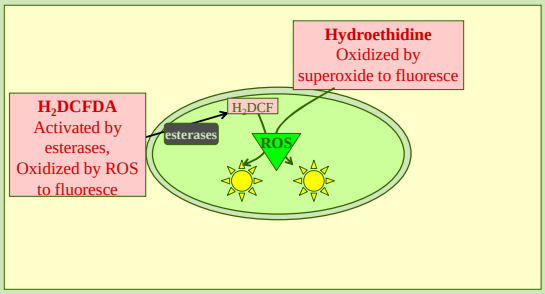
Detection of all types of ROS (O_2^1, $OH^{\cdot}$ and $O_2^{-\cdot}$)



Evidence of nC_{60}-mediated ROS damage in other organisms

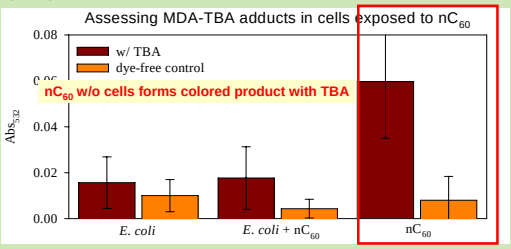
- Damage due to peroxidation of cell membrane by nC_{60}-mediated ROS production
- **Fish** (Oberdorster et al., *Environ Health Persp*, **2004**)
  - Some lipid peroxidation, no protein oxidation
- **Human cell lines** (Sayes et al., *Biomater*, **2005**)
  - Lipid peroxidation, increase in glutathione production, ascorbic acid afforded protection
- **Mammalian cell lines** (Isakovic et al., *Toxicol Sci*, **2006**)
  - Intracellular ROS detected, lipid peroxidation, protection by n-acetylcysteine

Does nC_{60} produce ROS in bacteria?



Looking for lipid peroxidation as evidence of ROS damage

- Hallmark of lipid peroxidation is malonedialdehyde (MDA)
- MDA forms colored adducts with thiobarbituric acid (TBA)



No Evidence of Oxidative Damage of Cytoplasmic Proteins

- An immunoassay was used to detect carbonyl groups (evidence of ROS damage) in cytoplasmic proteins.
- nC_{60} did not cause oxidative damage as compared to the control.

No conclusive evidence of ROS production or ROS-mediated damage

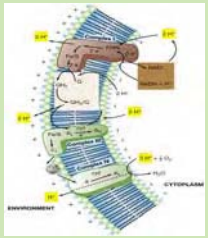
If not ROS, then how does nC_{60} exert its antibacterial effect?

nC_{60} is an oxidant

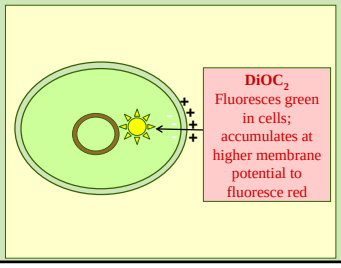
Substance	water	10 mg/L nC_{60}	1 M malic acid	1 M ferric chloride	1 M ferrous sulfate
ORP value (mV)	221-297	483	276	690	291

Could nC_{60} oxidize cell components or act as an uncoupler?

Membrane potential changes



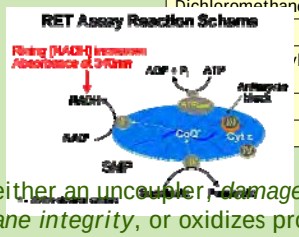
- Assay monitors $DiOC_2$
  - Red fluorescence indicates higher membrane potential
- Higher red/green ratio means higher membrane potential
- CCCP is an ionophore



Why membrane potential changes in Gram positive *B. subtilis* but not Gram negative *E. coli*?

Reverse Electron Transport (RET) hindered by nC_{60}

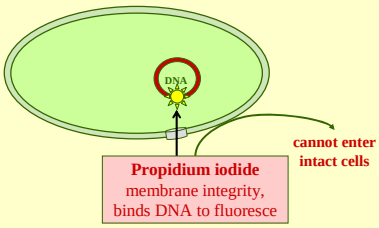
Chemical	EC_{50} (mg/L)
Chloramphenicol	165
Dichloromethane	33.7
	2.2
	0.713
	0.62
	0.14



- nC_{60} is either an uncoupler, *damages membrane integrity*, or oxidizes proteins

Does nC_{60} puncture cells?

- Propidium iodide enters permeablized cells and stains nucleic acids



What is the antibacterial mechanism of nC_{60}?

- No conclusive evidence of ROS production or ROS damage
  - Re-evaluate previous results based on the ability of nC_{60} to interfere with assays
- nC_{60} oxidizes bacteria
  - uncoupler
  - oxidize respiratory proteins

Acknowledgements

Lena Brunet, Laura Adams, David Brown, George Hinkal

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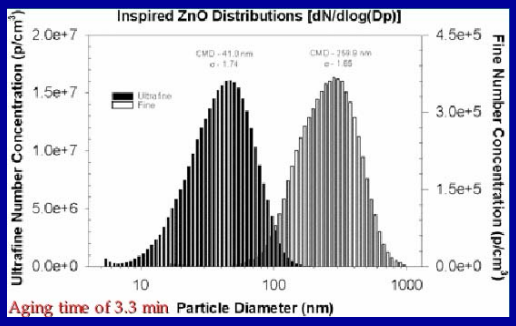
Role of Particle Agglomeration in Nanoparticle Toxicity

Terry Gordon, PhD
NYU School of Medicine

Study Hypothesis

- There is a difference in the toxicity of fresh (predominantly singlet) vs. aged (predominantly agglomerated) carbon nanoparticles
- This difference also applies to metal nanoparticles

Feasibility?



Beckett et al., Blue Journal, 2005

Objectives

1. Measure the agglomeration rate of carbon nanoparticles
2. Identify whether agglomeration is affected by altering exposure conditions such as humidity and particle charge
3. Compare the toxicity of singlet vs. agglomerated particles in mice exposed via the inhalation route

Experimental Approach

- Establish the agglomeration of freshly generated carbon nanoparticles at various distances (i.e., aging times) downstream from particle generation in a dynamic exposure system
  - Generated with a spark furnace
- Expose mice to nanoparticles at different stages of particle agglomeration
  - Expose to singlet and agglomerated at same time
  - Lungs will be examined for injury and inflammation
- Are findings for carbon nanoparticles applicable to other nanoparticles?
  - Generate zinc and copper nanoparticles

Methods

- Generate nanoparticles with Palas generator using Argon
- Dilute particle stream with air (supplemented with oxygen) and split into 2 paths: fresh and aged
- Expose mice for 2 to 5 hrs to filtered air or carbon, zinc, or copper nanoparticles
  - gravimetric measurements
  - particle size - WPS scanner (TSI, Inc.)
- Examine lung lavage at 24 hrs after exposure

Aged vs. Fresh Carbon Nanoparticles

- Low, middle, and high concentrations = 1, 2.5, and 5 mg/m^3
- Fresh = 1.5 sec downstream of the particle generator (≈ 11 to 90 nm)
- Aged = 3 minutes downstream (190 to 250 nm)

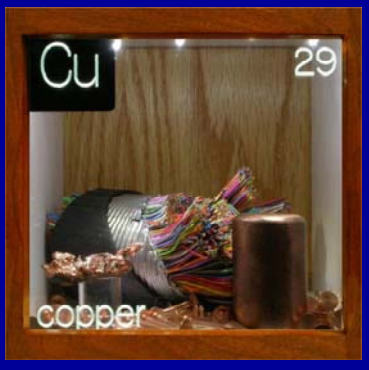
Data Presented Last Year

- Fresh vs. Aged carbon nanoparticles
  - Dose-response from 1 to 5 mg/m^3
- No difference in response with low or high humidity
- Particle charge had no effect

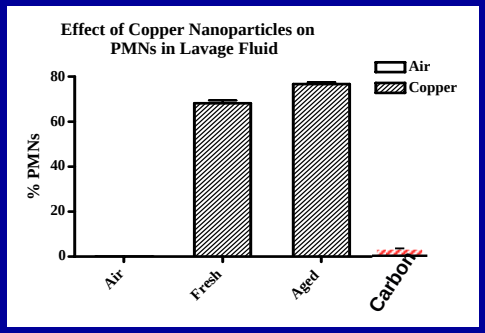
Effect of Other Nanoparticles?

- Copper
- Zinc

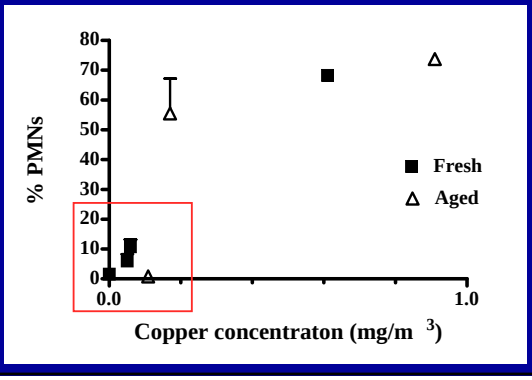
Effect of Other Nanoparticles?



Copper Nanoparticles (0.8 mg/m^3)



Fresh Copper Nanoparticles Effect on % PMNs

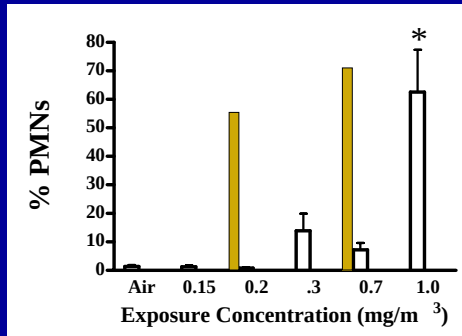


Fresh Copper Nanoparticles Effect on Protein

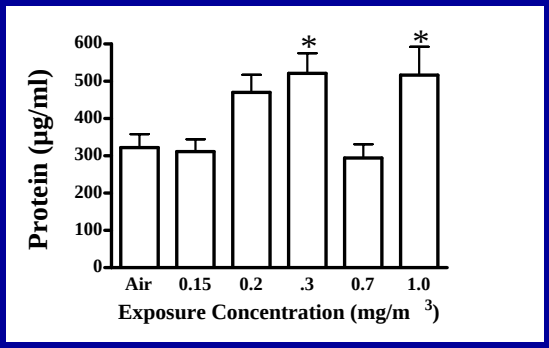
- Same general dose-response as for PMNs

Effect of Other Nanoparticles?

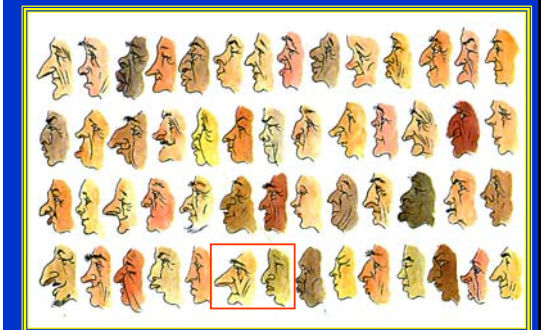
Fresh Zinc Nanoparticles Effect on PMNs

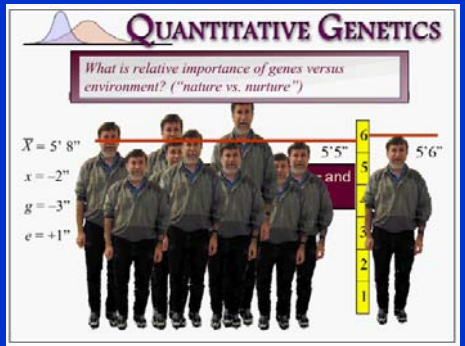


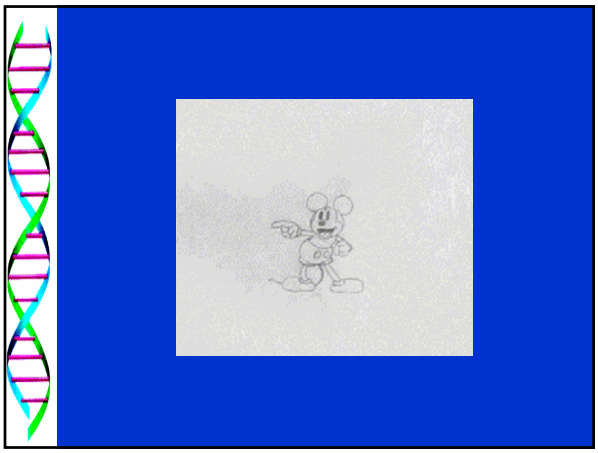
Fresh Zinc Nanoparticles Effect on Protein

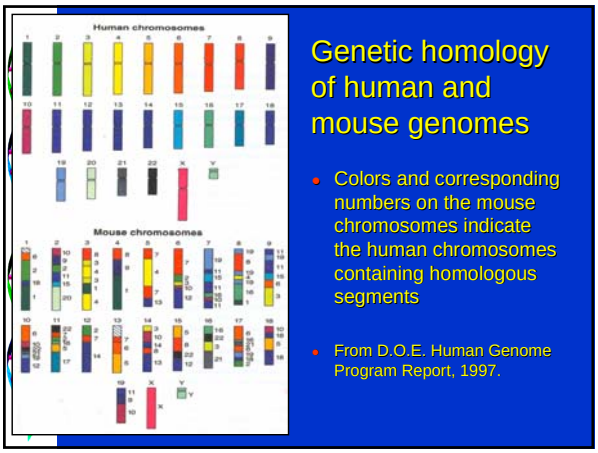


Do All Humans Respond the Same?









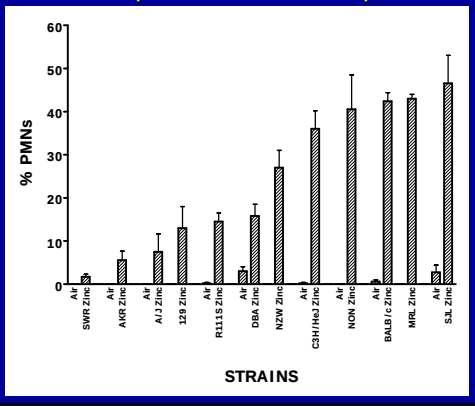
Genetic homology of human and mouse genomes

- Colors and corresponding numbers on the mouse chromosomes indicate the human chromosomes containing homologous segments
- From D.O.E. Human Genome Program Report, 1997.

Strain Response

- 2 hr exposure to 0.6 to 0.8 mg/m^3 fresh zinc nanoparticles
- 12 inbred strains of mice
  - BALB/c
  - MRL
  - SJL
  - AKR
  - NON
  - NZW
  - C3H/He
  - A/J
  - R111
  - 129
  - SWR
  - DBA

Strain Response to Zinc Nanoparticles



Conclusions

- Dose-response relationship between exposure to carbon and metal nanoparticles and lung inflammation
  - Fresh >> Aged effects for one type of particle (carbon) but not for others (copper)
- Humidity and charge had no effect on the toxicity of carbon nanoparticles

Conclusions (cont….)

- Copper and zinc nanoparticles
  - more toxic than carbon nanoparticles
  - Unlike with carbon nanoparticles, copper had only a small difference between fresh and aged nanoparticles
  - Copper nanoparticles were more toxic than zinc nanoparticles
- Strain differences in response suggest that genetic susceptibility could be involved in the response to nanoparticles

This research is funded by
U.S. EPA - Science To Achieve Results (STAR) Program
Grant # RD-8325280

Thanks to:

Nick Halzack, Karen Galdanes, Maire Heikkinen, and Judy Xiong, Lung Chi Chen, Beverly Cohen, Martin Blaustein

Transport and Retention of Nanoscale C-60 Fullerene Aggregates in Water-Saturated Soils

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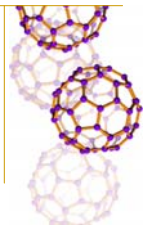

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Background

- Fullerene-based nanomaterial production is rapidly expanding
- Potential Toxicity: Lipid peroxidation, oxidative stress, reactive oxygen species (ROS)
- C_{60} forms stable nanoscale aggregates in water:
  - Aggregate diameter: 95-200 nm
  - Size and stability is dependent upon ion strength
- Limited research on n-C_{60} transport in porous media; high velocities, small columns, no retention profiles
- Classical filtration theory used to describe n-C_{60} transport and retention

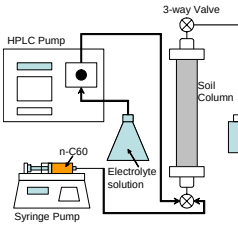
Video of n-C_{60} Aggregate Suspension (dia. ~95 nm, 1.0 mM $CaCl_2$, ~0.3 mg/L)

Research Objectives

1. Investigate the transport and retention of n-C_{60} aggregates in water-saturated soils as a function of soil properties and systems parameters.
2. Assess the effects of n-C_{60} aggregates on soil water retention, water flow and transport in unsaturated soils.
3. Develop and evaluate a numerical simulator(s) to describe n-C_{60} aggregate transport, retention and detachment in subsurface systems.

Experimental Methods: Column Studies

- n-C_{60} suspensions: THF+H_2O; 95-120 nm dia., ~ 3.0 mg/L
- Aqueous phase: 1.0 mM $CaCl_2$ + 0.065 mM $NaHCO_3$ or DI
- Pulse width: 3-10 dimensionless pore volumes
- At least two replicates per experiment (repacked column)



Ottawa Sand	Mean Diameter (mm)	Pore Velocity (m/d)
20-30 Mesh	0.71	~ 8.0
		~ 1.0
40-50 Mesh	0.35	~ 8.0
		~ 1.0
80-100 Mesh	0.16	~ 8.0
		~ 1.0
100-140 Mesh	0.13	~ 8.0
		~ 1.0

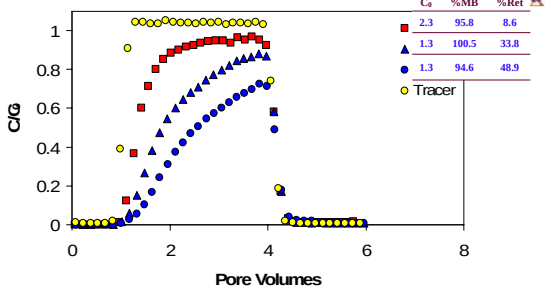
Experimental Study 1

Constant Grain Size and Flow Rate

- 40-50 mesh glass beads (GB) or Ottawa Sand (OS), d_{50}=0.33 mm
- n-C_{60} suspensions: 95 nm dia., 1.3 or 2.3 mg/L
- Aqueous phase: 1.0 mM $CaCl_2$ + 0.065 mM $NaHCO_3$ or DI
- n-C_{60} zetapotential (mV): -29.2 (1 mM $CaCl_2$); -63.98 (0 IS)
- Pore-water velocity (v_p) ~ 8.0 m/d; Flow rate, (Q) ~ 1.0 mL/min.
- Experimental Sequence:

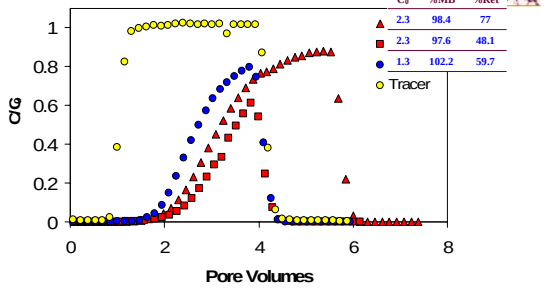
Tracer test → 3 or 5 PV C_{60} injection → 3 PV water elution → Column dissection

n-C_{60} Effluent BTCs 40-50 mesh Glass Beads



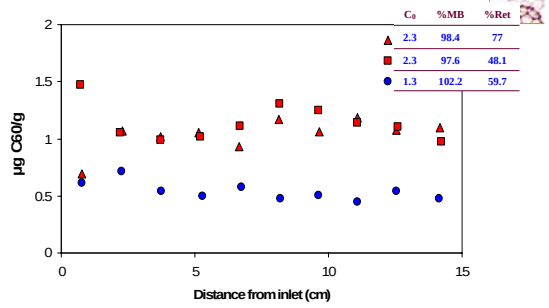
θ = 0.38; IS = 1.0 mM $CaCl_2$; PW = 3 pv; Q = 1.0 mL/min

n-C_{60} Effluent BTCs 40-50 mesh Ottawa Sand



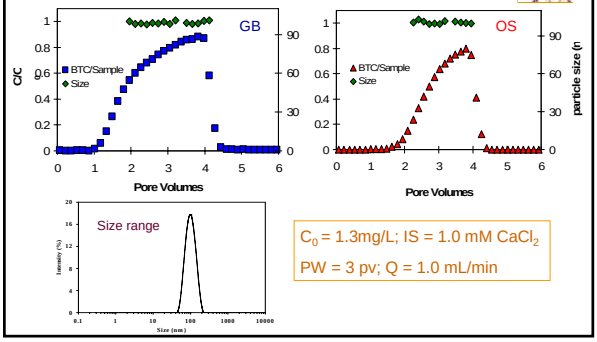
θ = 0.36; IS = 1.0 mM $CaCl_2$; PW = 3 or 5 pv; Q = 1.0 mL/min

n-C_{60} Retention Profiles 40-50 mesh Ottawa Sand



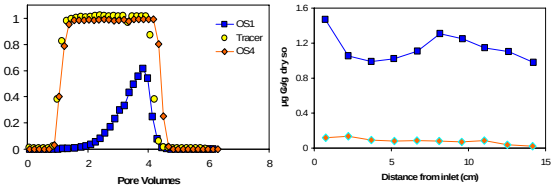
θ = 0.36; IS = 1.0 mM $CaCl_2$; PW = 3 or 5 pv; Q = 1.0 mL/min

Did n-C_{60} Aggregate Size Change?



C_0 = 1.3mg/L; IS = 1.0 mM $CaCl_2$
PW = 3 pv; Q = 1.0 mL/min

Effect of Ionic Strength (IS) 40-50 mesh Ottawa Sand



	C0	IS	PW	%MB	%Ret
OS1	2.3	1mM	3	97.6	48.1
OS4	2.3	0	3.25	100.2	4.8

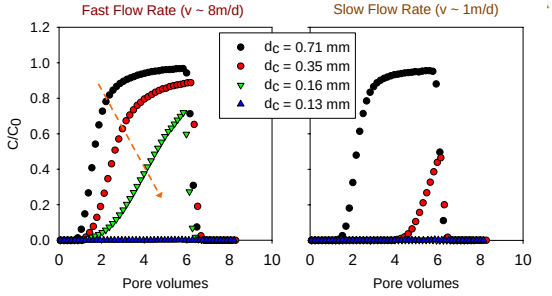
Zeta Potential: -29.2 mV (1mM CaCl2) or -64.0 mV (DI)

Experimental Study 2

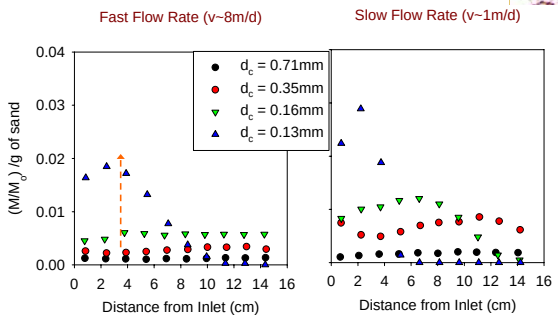
Effect of Grain Size and Flow Rate

- n-C_{60} suspension: ~120nm, ~3.0mg/L (5 or 10 pore volumes)
- Four size fractions of Ottawa sand:
  20-30 (0.71mm), 40-50 (0.35mm), 80-100 (0.16mm), 100-140 (0.13mm)
- Two Pore-water Velocities: v_p = 1 or 8 m/d
- Two Electrolyte Conditions: 1mM $CaCl_2$ + 0.065mM $NaHCO_3$ or DI water
- Total of 22 column experiments

Selected n-C_{60} Effluent BTCs: Effect of grain size and flow rate



Selected n-C_{60} Retention Profiles: Effect of grain size and flow rate

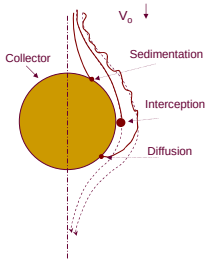


Mathematical Modeling

- Filtration Model

$$\frac{\partial C}{\partial t}+\frac{\rho_b}{\theta}\frac{\partial S}{\partial t}=D_h\frac{\partial^2 C}{\partial x^2}-v_p\frac{\partial C}{\partial x}$$

$$\frac{\rho_b}{\theta}\frac{\partial S}{\partial t}=k_{att}C \qquad k_{att}=\frac{3(1-\varepsilon)v}{2d_c}\alpha\eta_0$$



Dispersion is often ignored:

$$S(x)=\frac{t_0 \varepsilon k C_0}{\rho_b}\exp\left(-\frac{k}{v}x\right)$$

Yields exponential retention profiles

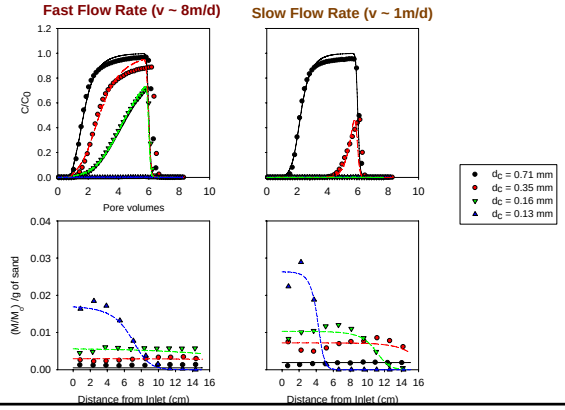
$$\frac{\rho_b}{\theta_w}\frac{\partial S}{\partial t}=k_{att}\Psi C-\frac{\rho_b}{\theta_w}k_{det}S$$

$$\Psi=\frac{S_{max}-S}{S_{max}}$$

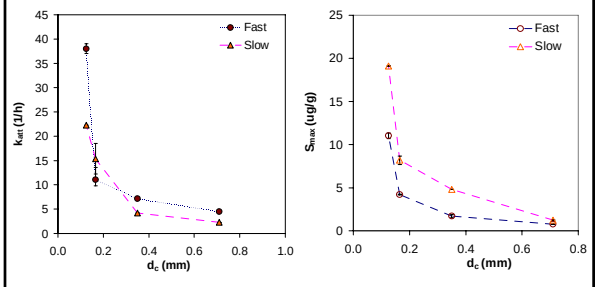
Flat retention profile

(Johnson and Elimelech, 1995)

Simulation Results

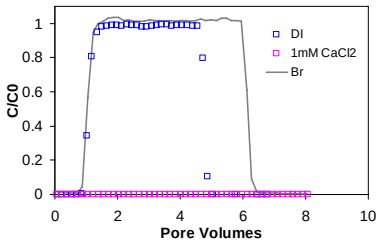


Fitted Parameters k_{att} and S_{max}



Low Ionic Strength Experiments

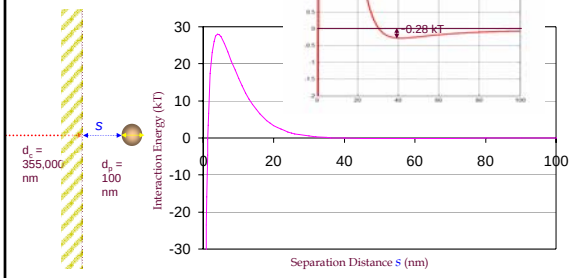
- When IS → 0, n-C_{60} and tracer BTCs are identical even in 100-140 mesh OS



C_0 = 3 mg/L; PW = 3.8 or 5 pv; v_p = 8 m/d

* **Retention is electrostatic in origin**

Secondary Minimum



$Ø_{n\text{-}C60}$ = - 29.2mV; $Ø_{sand}$ = - 53mV; Ionic Strength = 1mM

Secondary Minimum

- Compare with Hydrodynamic Drag Force

Interaction force due to secondary minimum >> **Hydrodynamic drag force**

F_A = 2.4E-14 N (Israelachvili, 1992)

F_D = 2.43E-16 ~ 1.12E – 15 N (Payatakes et al., 1973)

- Theoretical Attachment Rate k_{att}

$$k_{att} = \frac{3(1-\varepsilon)v}{2d_c}\alpha\eta_0$$

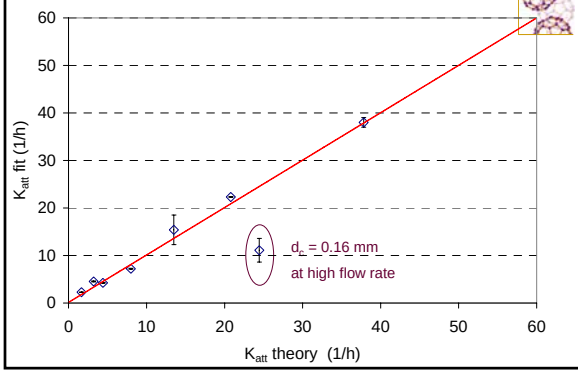
- Collision efficiency -- the fraction of n-C_{60} particles that have energy less than the secondary minimum

$$\alpha = \int_0^{-\Delta G_{2nd\,min}} f(E_{kin})dE$$

- Maxwell distribution for frequencies of the kinetic energy

$$f(E_{kin}) = \frac{2}{\sqrt{\pi}kT}\sqrt{\frac{E_{kin}}{kT}}\exp\left(-\frac{E_{kin}}{kT}\right)$$ (Hahn and O'meliá, 2004)

Comparison of Theoretical and Fitted k_{att}



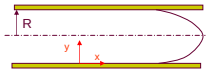
Retention Capacity S_{max}

- **Previous Work:**
  - S_{max} = *f* (water chemistry, surface potential) (Adamczyk et al., 1994)
  - S_{max} is influenced by "Shadow Effect" created by shear component of the fluid flow around collector grains. (Ko and Elimelech, 2000)
  - Difficult to quantify S_{max} a priori (e.g., batch experiments)
- **Our Observations** (with fixed water chemistry)**:**
  - Diffusion is the dominating mechanism for n-C_{60} nanoparticle transport $\eta \approx \eta_{diffusion}$
  - Fitted S_{max}= *f* (flow intensity, particle size)

Influence of diffusional boundary layer?

Mass Flux in Diffusional Boundary Layer

- Flow in a Pore Tube



$$\begin{cases} \frac{2v_0}{R}y\frac{\partial C}{\partial x} = D\frac{\partial^2 C}{\partial y^2} \\ C \to C_o \quad @\ y \to \infty \\ C = 0 \quad @\ y = 0 \end{cases}$$

$$j_{diff} = D\left(\frac{\partial C}{\partial y}\right)_{y=0} = \frac{DC_0}{\delta}$$

Diffusional Boundary Layer Thickness :

$$\Rightarrow \delta = \frac{1}{0.67}\left(\frac{D}{v_o R}\right)^{1/3}(R^2 x)^{1/3}$$

Mass flux to the surface :

$$I_{diff} = 2\pi R\int j_{diff}dx$$

$$\Rightarrow I_{diff} = 2.01\pi C_o D\left(\frac{v_0 x^2}{DR}\right)^{1/3} R \quad [M/T]$$

Normalized Mass Flux:

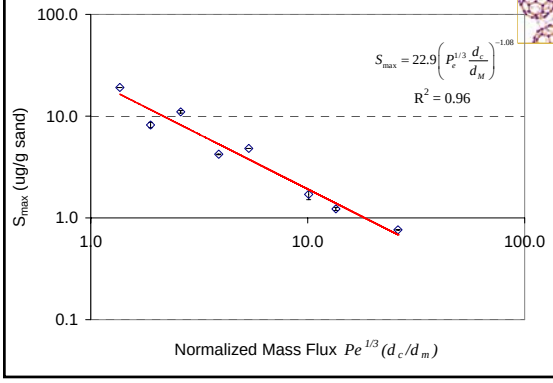
$$\Rightarrow \Lambda = \frac{I_{diff}}{DC_0 d_M} \qquad x, R \gg d_c$$

$$\Rightarrow \Lambda \sim \left(\frac{v_o d_c}{D}\right)^{1/3}\frac{d_c}{d_M} = P_c^{1/3}\frac{d_c}{d_M}$$

d_M: mean diameter of a medium sand, 0.05 cm

Correlation between S_{max} and Normalized mass flux

Fitted S_{max} and Normalized Mass Flux



Conclusions

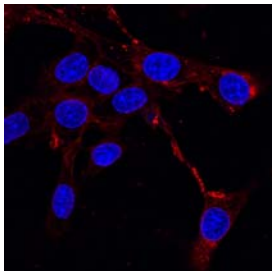
- n-C_{60} aggregate transport decreases and retention increases as grain size or flow rate are decreased.
- Detachment rate coefficient approached 0, and did not change with grain size or flow rate.
- A mathematical model that includes non-equilibrium, non-linear retention captured n-C_{60} transport and retention behavior in Ottawa Sands.
- n-C_{60} aggregate transport and retention is strongly influenced by IS; importance of electrostatic interactions.
- Secondary minimum plays an important role in n-C_{60} attachment.
- n-C_{60} retention capacity was correlated with mass flux in diffusional boundary layer.

Future Work

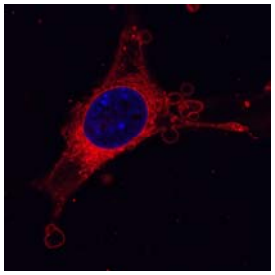
- Measure and simulate n-C_{60} transport and retention in a water-saturated “natural” soil(s) (e.g., Appling soil).
- Measure and simulate n-C_{60} transport and retention in unsaturated porous media.
- Investigate the effect of stabilizing/dispersive agents (e.g., NOM, surfactants) on n-C_{60} transport and retention in Ottawa Sands (Hyung et al., 2007, *ES&T*, 41:179-184).
- Determine THF and γ-butyrolactone (GBL) concentrations in purified and unpurified n-C_{60} suspensions (Henry et al., 2007, *EHP*, 115:1059-1065).
- Evaluate neurotoxicity of manufactured nanomaterials.

Confocal Images of Neuronal Cells Treated with Rhodamine-labeled Iron Particles

40X



100X



Impacts of Fullerene (nC60 or C60) on Microbiological Functions in Soil and Biosolids

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Zhonghua Tong
Larry Nies
Leila Nyberg
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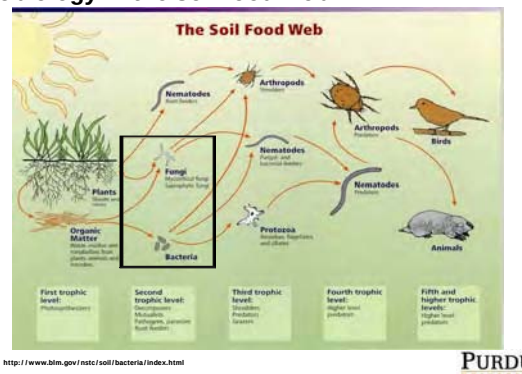
PURDUE

Our question is whether C60 is impacting the microbiology in the soil food web



http://www.blm.gov/nstc/soil/bacteria/index.html

2

PURDUE

The talk presents the findings from a number of ongoing projects

Soils Work

Biosolids Work

Fungal Work

3

PURDUE

Soils are typical of the Midwest and chemical C60 preparations methods are established

Name	OM		Texture		
			Sand	Silt	Clay
	%	pH		%	
Drummer	3.6	6	17	52	31
Tracy	1.5	5.5	55	37	8

Formation: Deguchi, et al., 2001
Concentration: Fortner et al., 2005
Size: DLS system

4

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Our chosen microbiology methods are well established and documented

Evaluate Microbial Systems

Microbial Form
- PLFA
  - Biomass Size

PCR-DGGE
- Three domain model

Functions (CO_2 CH_4)

Glucose Assimilation (^{14}C-CO_2)

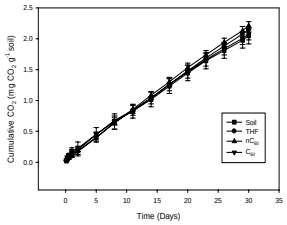
Fungal Abilities (^{13}C)

5

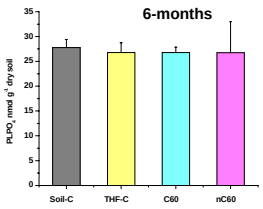
PURDUE

C60 and nC60 had little impact on soil functions

Soil Respiration



Biomass Size



nC60 1 ppm / C60 1000 ppm – Drummer Soil

6

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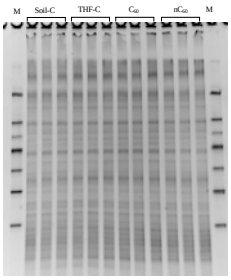
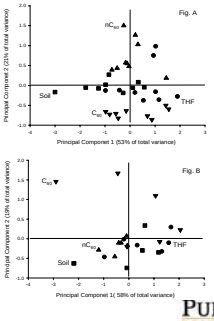
Microbial profiling showed no difference after six months

DGEE – 6 months



PLFA 3 or 6 Months

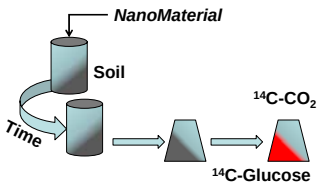


PURDUE

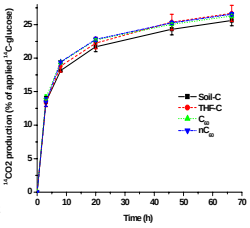
No impact from longer incubations – Glucose assimilation testing method established

Test procedure

Soil Challenged
Soil Incubated
Soil Tested



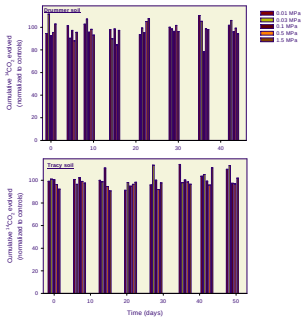
Response at 6 months



PURDUE

Combinations of fullerenes with soil water stress show no effects

- Five water potentials
- Two nano materials
  (nC60, C60, C12)
- Two Soils
- 50 Day Incubation
- Respiratory response

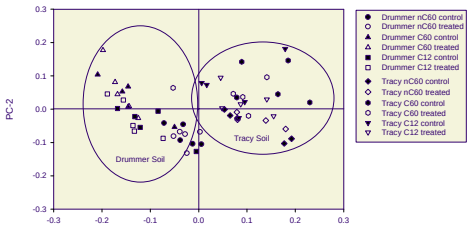


Microbial activity measured as $^{14}CO_2$ evolved from ^{14}C-glucose treated soil exposed to nC60.

PURDUE

Soil diversity showed no effects from C60 combined with water potential

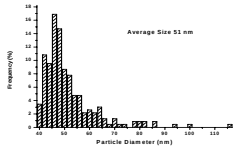
Fatty Acids patterns from soils with nanomatrials and under water stresses (each symbol has an associated water potential)

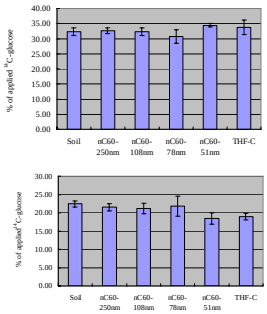


PCA developed from FAMEs for treated and untreated soil.

PURDUE

Preliminary data suggests nC60 crystal size has no effect on soil response





- nC60 formed in different size classes (mixing speed) added to soil
- Respiratory response after 30 day exposure

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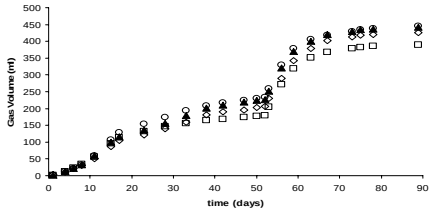
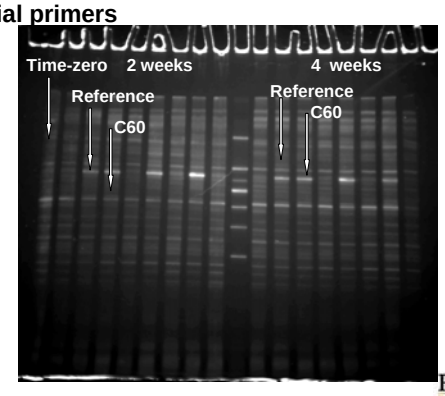
Biosolids -- (Anaerobic Systems) are not impacted by C60

Biosolids system & C60 (50,000 ppm)

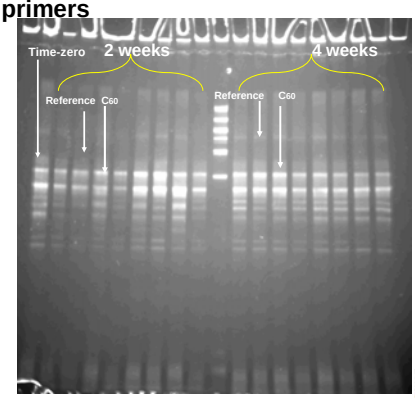


PURDUE

No impact on community structure tested with bacterial primers

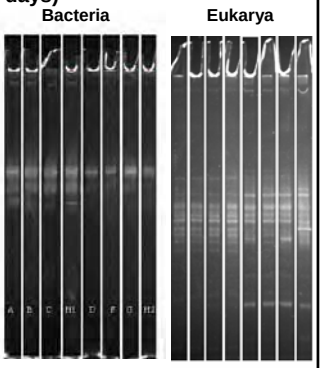


No impact on community structure tested with Archaea primers



C60 type and concentration showed no effect on anaerobic system (150 days)

Sample ID	C_{60} (mg/kg biomass (d/w)	Fullerene Prep
A	0.321	Dissolved MeOH/EtOH
B	8.6	aq-C_{60}
C	30,000	Plated on dried sludge (toluene)
D	50,000	Plated on dried sludge (*o-xylene*)



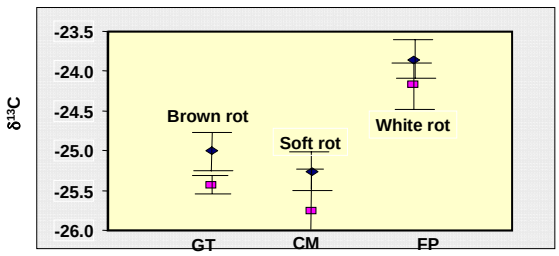
Tracking Fungal utilization of C60 requires $^{13}C60$

Fungal Species
Gleophyllum trabeum
Fomitopsis pinicola
Cadophora malorum

^{13}C-C_{60} added 150 µg C60 (15 atom %)
Growth media
Wood blocks

Tracking ^{13}C in Biomass and Headspace Gases

Averages of 13C/12C ratios fungi grown on wood blocks were not all that different for the two materials.



Gleophyllum trabeum
Fomitopsis pinicola
Cadophora malorum

C60-OH and fungi is under investigation

In summary, C60 and nC60 have had limited impact on microbiology of soil and biosoilds. Transformation of C60 by fungi is also limited.

Soil biomass size and structure unchanged
- repeat applications and solvent effects under investigation

Biosolids biomass size and structure unchanged
- functional groups are being investigated

Fungal utilization of C60 not apparent
- work on C60-oL is on going.

PURDUE

Funding Support

Funding support from National Science Foundation Award EEC-0404006 & US EPA Award RD-83172001-0

PURDUE

Size Distribution and Characteristics of Aerosol Released from Unrefined CNT Material

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NIEM
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Carbon Nanotubes

Carbon nanotubes represent a new form of carbon that has closed tubular structures, consisting of nested cylindrical graphitic layers with a hollow internal cavity and capped by fullerene-like ends (Iijima 1991).

Carbon nanotubes offer a full range of electrical and thermal conductivity properties, are about a hundred times stronger than steel, and are more durable than diamonds.

Safety and Health Aspect

- Carbon nanotubes (CNTs) are among the most dynamic and fast-growing nanomaterials due to their novel properties.
- As production rate scaling up, the potential of human exposure to this new type of materials in workplace as well as in the general environments are rising.
- Their impacts on human health are of great concern by many researchers.

Inhalation Exposures

- To determine the overall risk to human and environment, not only the material toxicity but also the exposure levels need to be considered.
- For many conventional workplace contaminants, airborne route is considered the most crucial for worker protection.
- To determine the worker exposure levels to airborne nanoparticles, the particle concentration, size distribution, shape characters, as well as the agglomeration status are among the main factors.

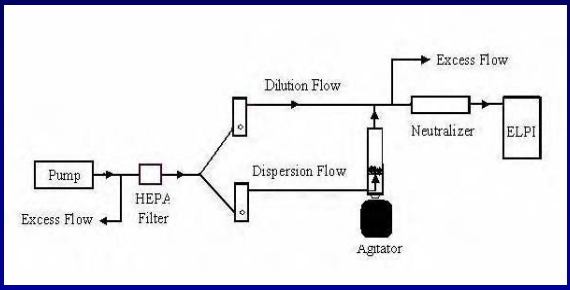
Characteristics of CNTs

- High aspect ratio (typically in a scope of 10^2 but can reach as high as 10^4).
- Highly agglomerated.
- Often coexist with non-tubular type particles, such as amorphous carbon soot, metal catalysts as well as ambient particulate matters.
- The size distributions of CNTs are hard to predict, but presumably widely spread and source dependent.

Specific Aims

- To investigate the size distribution and characteristics of aerosol particles released from various types of industrial grade CNT bulk materials due to agitation. The results will provide a foundation for developing field and personal sampling devices for CNTs.
- To develop a practical method using atomic force microscopy image analysis that is capable to classify CNTs from other co-existing nano-sized particles in general environments.
- To develop appropriate methods for monitoring the potential worker exposure levels to CNTs.

Test Aerosol Generation



Sample Material Sources

- The size dimensions of CNTs vary with the type and the methods by which they are manufactured. Therefore, size distributions of CNT aerosol particles also depend on the source of the material.
- Up-to-date 7 Industrial-grade bulk CNT samples from 3 manufacturers have been examined.
- The sample matrix includes 3 common types of CNTs, i.e., single-walled, double-walled and multi-walled, and 3 primary methods of production, i.e., arc discharge (Arc), chemical vapor deposition (CVD), and high-pressure CO conversion (HiPco).

Methods

Airborne Particle Sampling and Sizing:

- Electrical Low Pressure Impactor (ELPI)
- Integrated Screen Diffusion Battery (ISDB)

Particle Counting and Characterization:

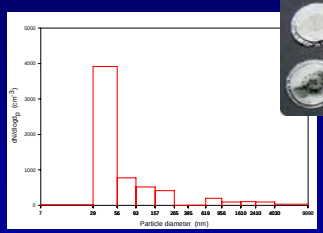
- Atomic Force Microscopy (AFM)

Calibrated Aerodynamic Cut-off Size of ELPI

Stage Number	Aerodynamic Cut-size (μm)
Filter	0.007
1	0.029
2	0.056
3	0.093
4	0.157
5	0.265
6	0.385
7	0.619
8	0.956
9	1.61
10	2.41
11	4.03
12	-
13	9.99

CVD-SWCNT Number Weighted Size Distribution

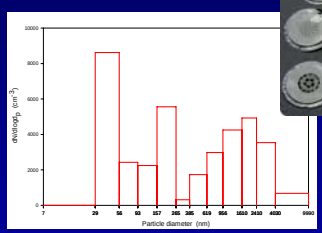
dp≤100 nm: ~80%



Sample: CVD-SWCNT
Grade: HP
Diameter: < 2 nm
Length: 0.5-40 μm
Purity: > 90%
Manufacturer: Helix, TX

Arc-SWCNT Number Weighted Size Distribution

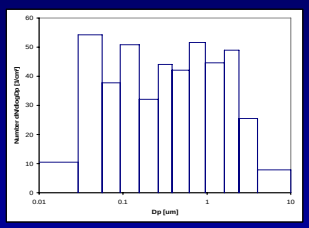
dp≤100 nm: 35%



Sample: Arc-SWCNT
Grade: AP
Diameter: 1.4 nm
Length: 2-5 μm
Purity: 50-70%
Manufacture: CarboLex, IN

CVD-SWCNT Number Weighted Size Distribution

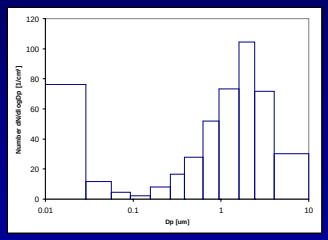
dp≤100 nm: 30%



Sample: CVD-SWCNT
Grade: LC
Diameter: 1.2-1.5 nm
Length: 0.5-3 µm
Purity: 50-70%
Manufacture: Helix, TX

HiPco-SWCNT Number Weighted Size Distribution

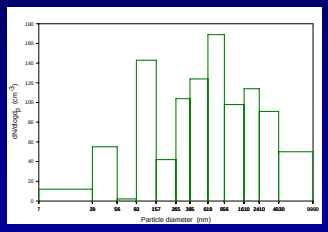
dp≤100 nm: ~3%



Sample: HiPco-SWCNT
Grade: AP
Diameter: 0.8-1.2 nm
Length: 0.1-1 µm
Purity: 65%
Manufacture: CNI, TX

CVD-DWCNT Number Weighted Size Distribution

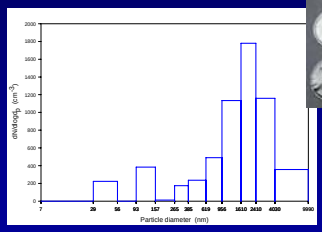
dp≤100 nm: < 20%



Sample: CVD-DWCNT
Grade: HP
Diameter: < 5 nm
Length: 0.5-40 µm
Purity: 90%
Manufacturer: Helix, TX

CVD-MWCNT Number Weighted Size Distribution

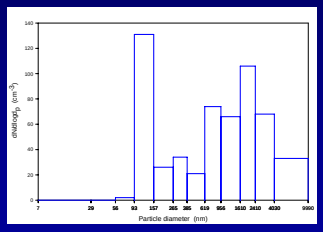
dp≤100 nm: < 5%



Sample: CVD-MWCNT
Grade: Short
Diameter: < 10 nm
Length: 1-2 µm
Purity: 95%
Manufacture: Helix, TX

CVD-MWCNT Number Weighted Size Distribution

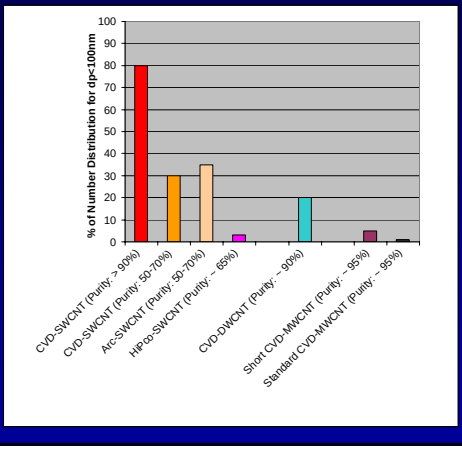
dp≤100 nm: 1%



Sample: CVD-MWCNT
Grade: Standard
Diameter: < 10 nm
Length: 0.5-40 µm
Purity: 95%
Manufacture: Helix, TX

Number Weighted 'Nano-size' Fraction of Airborne Unrefined Carbon Nanotubes

CNT Type	Grade	Manufacture Method	Manufacturer	Purity	Diameter (nm)	Length (µm)	Number Distribution for d_p < 100nm
Single-walled	HP	CVD	Helix, TX	>90%	<2	0.5-40	80%
Single-walled	AP	Arc	Carbolex, IN	50-70%	1.4	2-5	35%
Single-walled	AP	CVD	Helix, TX	50-70%	1.2-1.5	0.5-3	30%
Single-walled	AP	HiPco	CNI, TX	65%	0.8-1.2	0.1-1	3%
Double-walled	AP	CVD	Helix, TX	90%	<5	0.5-40	20%
Multi-walled	Short	CVD	Helix, TX	95%	<10	1-2	5%
Multi-walled	Standard	CVD	Helix, TX	95%	<10	0.5-40	1%

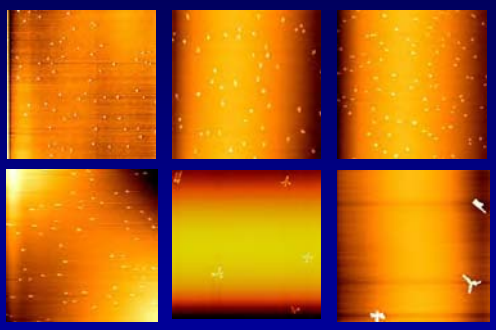


Integrated Screen Diffusion Batteries (ISDB)

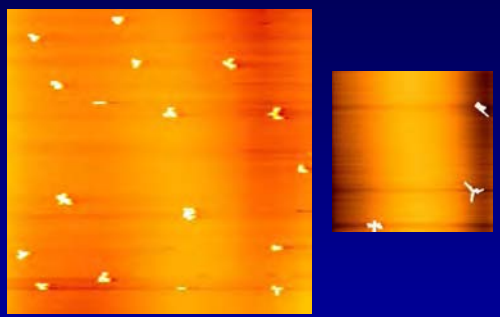
- This portable device was developed for collecting time-integrated samples of nano-sized particles, based on diffusional collection of particles on filtering elements and walls of a round tube (2 cm in diameter).
- The filtering elements used in this study are stainless steel wire screens in different mesh sizes (60 – 440).
- On the walls of the tube, between the filtering elements there are recessed slots for duplicate detectors. Mica discs were used as collectors.
- The sample collected on the mica discs can be analyzed directly by AFM.

- When particles are sampled into the tube the smallest particles, with highest diffusion coefficients, are collected first. Increasing number of bigger particles will be collected by the subsequent filtering elements.
- The collection efficiencies of the wire screens are calculated from equations presented by Cheng et al. (Cheng and Yeh 1980, Cheng et al. 1980, 1985).
- Particle size dependent deposition efficiency on the substrate is calculated with an equation developed by Ingham (1975).
- Particle size distributions are calculated using the Extreme Value Estimation deconvolution program (Paatero 1990).

AFM Images of a CVD-SWCNT Sample Collected on the Walls of a 6-stage ISDB



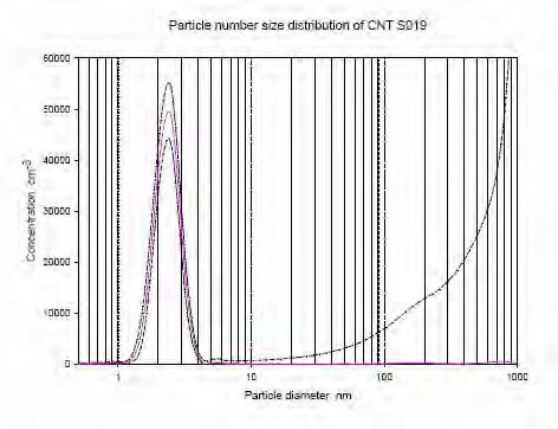
(Non-contact mode; Scan size: 25μm x 25μm,).



Non-contact mode AFM image of CVD-SWNT samples (HP grade, Helix,TX) on mica disc placed on the wall of ISDB stage 6: (a) 100μm x 100μm (b) 25μm x 25μm.

Counts of Particles Collected on the Wall of Each ISDB Stage

Stages	Particle Counts/10000μm2	
	mean	s.d.
1	1120	242
2	1584	156
3	1520	171
4	1000	102
5	76	7
6	46	13



Characterization of CNTs

- Sample Collection: ELPI (on Aluminum Discs placed on each stage) or ISDB (wire screens).
- Sample Analysis: Atomic Force Microscopy.
- Sample Preparation: Deagglomeration of samples by applying appropriate surfactant/solvent and sonication.

AFM Image of Deagglomerated SWCNT Samples

29 μg/ml CVD-SWCNT (HP grade, Helix, TX) deagglomerated by DMF [Scan size: 5μm x 5μm].

Summary of Experimental Results

- All common types of unrefined CNTs including single-walled, double-walled and multi-walled nanotube samples can be dispersed into air to a significant extent due to agitation.
- The sizes of particles generated from all CNT types are widely distributed across 13 stages of ELPI, ranging from 7 nm to 10 μm. The size distributions vary with the type and the nature of bulk materials.

- For HP grade CVD-SWCNT, majority of particles are in the nano-size region (< 100nm) based on the ELPI data. There is also a significant portion of particles found in the single-nanometer range based on the data collected by ISDB.
- Airborne CNT particles are highly agglomerated; no single tubes or simple ropes were observed by AFM in the original samples collected by ELPI or ISDB before treatment with surfactants.

Implications

- Carbon nanotubes can become airborne and expose workers through inhalation or dermal contact during the processes of manufacturing and handling.
- The size distributions of CNTs are wide and source dependent.
- As deposition efficiency and sites of inhaled particles within the respiratory system largely depends on particle size, the deposition pattern of agglomerated nanoparticles should be similar to those larger equivalent sized non-agglomerated particles.

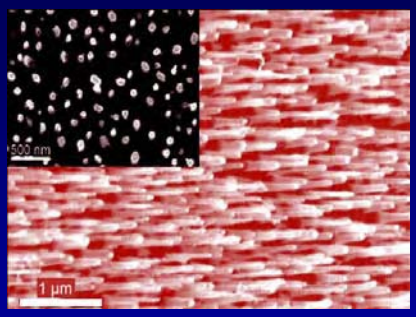
- Particles depositing on/in the deep lung surfaces of the bronchioles or alveoli will contact pulmonary surfactants in the surface hypophase and the agglomerated CNTs are likely to (ultimately) be de-agglomerated.
- Investigations that define CNTs should take into account the full size range of particles to which humans are likely to be exposed.
- Adequate monitoring methods need to be established for quantification and characterization of these new types of materials in order to evaluate the worker's exposure levels and hence the potential health risks.

On-going Studies

- Developing a quantitative sample treatment method for AFM analysis that can effectively deagglomerate samples by applying appropriate surfactants, solvent, and sonication.
- Exploring other advanced AFM technologies that may be better suited for CNT characterization, such as, Conductive-AFM and Phase Imaging.
- Developing a validated field sampling method for airborne CNT particles in workplaces.

Acknowledgements

- This study was supported by U.S. National Institute for Occupational Safety and Health (NIOSH) under Grant 5-R01-OH008807.
- Partial support was supplied by National Institute of Environmental Health Sciences (NIEHS) under Grant ES-0260.



This image from NASA-Ames shows an American flag made from carbon nanotubes using a plasma carbon vapor deposition technique.

The stripes are side views of the tubes which measure one micron in length. The "stars" against the blue field are the nanotubes viewed from the top.

Thank You!

Physical and Chemical Determinants of Carbon Nanotube Toxicity

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Agnes B. Kane, M.D., Ph.D.
Department of Pathology and Laboratory Medicine

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OUTLINE

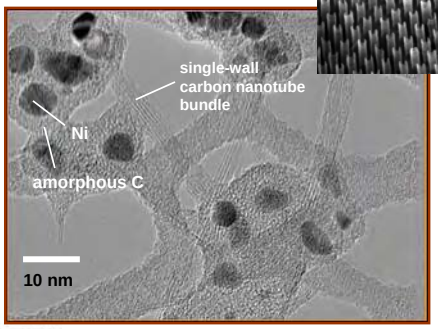
1. Bioavailability of nanotube metal residues
2. Adsorption of essential micronutrients by nanotubes and implications for toxicity testing

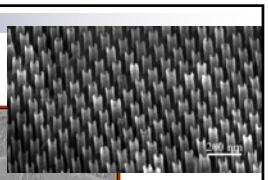
on toxicity mechanisms

3. TPGS as a safe, antioxidant surfactant for green nanotube processing
4. Targeted removal of bioavailable metal as a nanotube detoxification strategy

on toxicity management, or "green" nanomaterials

Many Nanomaterial Samples are Complex



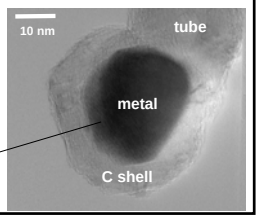


Ideal nanotube structure (J. Xu et al.)

Actual nanotube structure (commercial, as-produced)

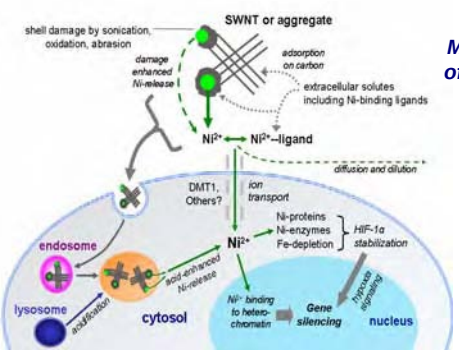
1. Bioavailability of Nanotube Metal Residues

- Catalytic growth methods:
  - now dominant for synthesis of multiwall nanotubes (esp. large scale)
  - only route for single-wall nanotube synthesis
- Most common elements in CNT catalyst formulations are Fe, Ni, Y, Co, Mo
- Ultrafine metals pose documented inhalation health risks depending on form, exposure route, dose
- Do metals contribute to CNT toxicity? How can we assay for and manage CNT metals effects?



Bioavailability? (the key issue)

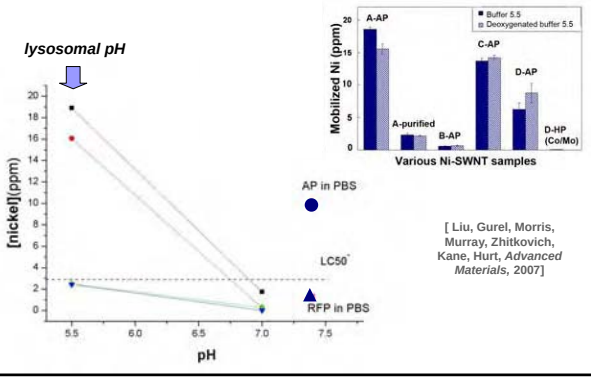
Molecular Mechanisms of Ni Toxicity



[Liu, Gurel, Morris, Murray, Zhitkovich, Kane, Hurt, *Advanced Materials*, 2007]

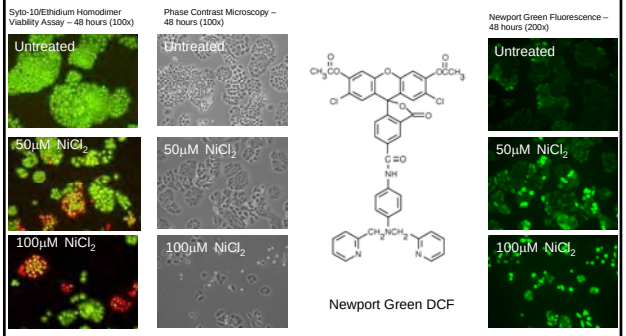
Ni ion hypothesis: **Ni toxicity, carcinogenicity depend on intracellular Ni^{2+} pool**

Nickel Mobilization from SWNTs: Effect of Media and Sample Origin



[Liu, Gurel, Morris, Murray, Zhitkovich, Kane, Hurt, *Advanced Materials*, 2007]

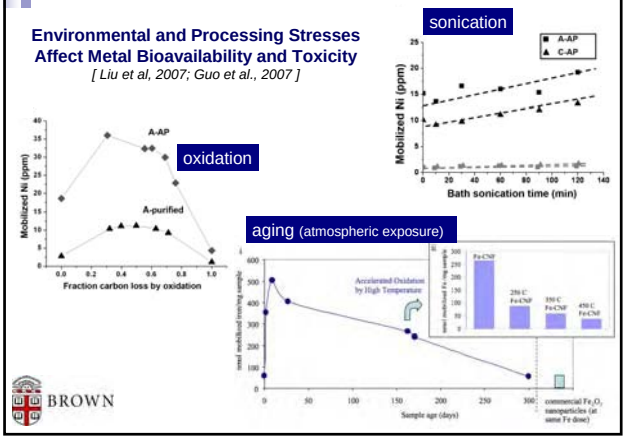
Cytotoxicity and Cellular Uptake of $NiCl_2$



Newport Green DCF

Environmental and Processing Stresses Affect Metal Bioavailability and Toxicity

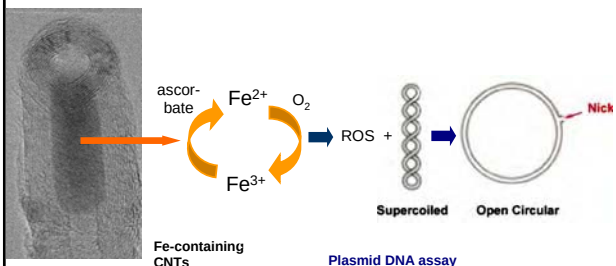
[Liu et al, 2007; Guo et al., 2007]



BROWN

CNTs show redox activity through release of bioavailable iron

[Guo., Morris, Liu, Vaslet, Hurt, Kane, *Chemistry of Materials*, 2007]



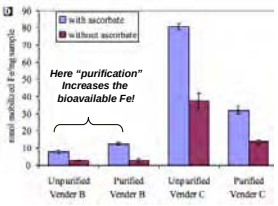
Fe-containing CNTs

Plasmid DNA assay
Single-strand break Induces uncoiling event detectable by gel electrophoresis

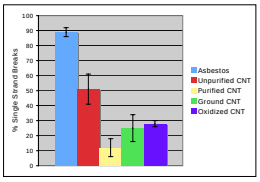
BROWN

Iron Bioavailability and Redox Activity of Diverse Carbon Nanotube Samples

From Guo et al., Chemistry of Materials 2007



Fe mobilization



DNA single-strand breaks

2. Adsorption of essential micronutrients by nanotubes and its implications for toxicity testing

Carbon nanomaterials observed to interact with molecular probe dyes

- MTT assay for cell viability gives false indication of CNT cytotoxicity due to interaction with MTS metabolite dye

  -- Worle-Knirsch et al. *Nano Lett.*, **6** (6): 1261 -1268, 2006

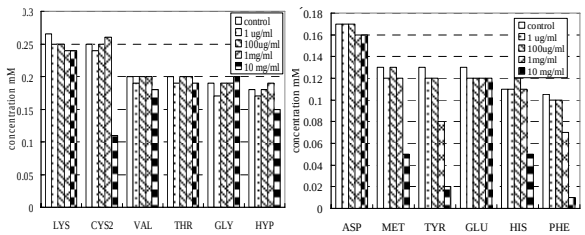
- Various indicator dyes are unsuitable for quantitative toxicity measurement

  -- Casey et al. *Carbon*, 45: 34-40 2007

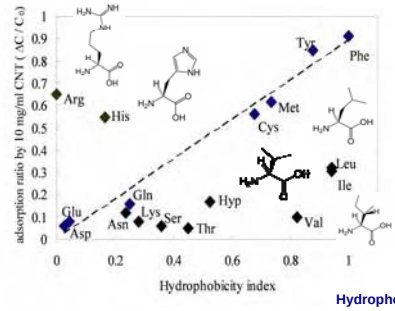
- Even carbon black (negative control) can influence biological assays

  -- Monteiro-Riviere et al. *Carbon*, **44** (6):1070-1078, 2006

Dose-dependent CNT adsorption of amino acids from cell culture media



Correlation of amino acid adsorption with hydrophobicity



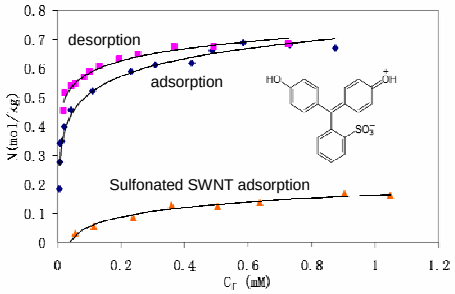
Molecular properties favoring adsorption on carbon:

- Hydrophobicity
- $\pi-\pi$ interactions
- Positive charge

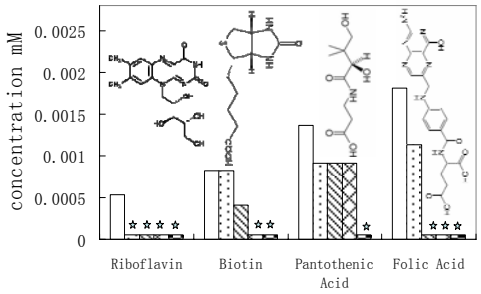
Hydrophobicity index of Black and Mould, 1991

Depletion mechanism can be studied through single-component experiments

adsorption Isotherms for phenol red



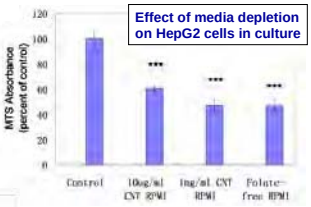
Some vitamins are depleted at CNT doses as low as 10 ug/ml



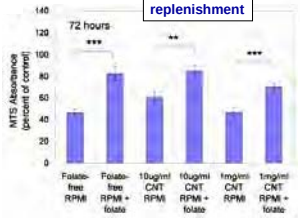
control 0.01mg CNT/ml 0.1mg CNT/ml 1mg CNT/ml 10mg CNT/ml

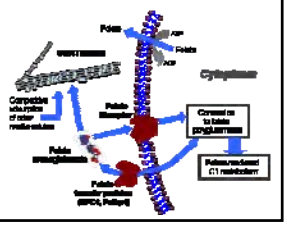
Biological implications of media depletion

Effect of media depletion on HepG2 cells in culture



Effect of folate replenishment

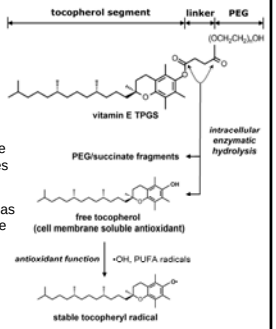




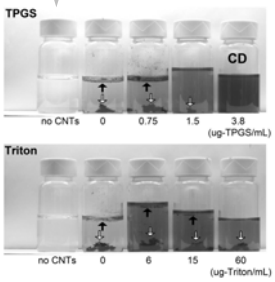
3. TPGS as a safe, antioxidant surfactant for green CNT processing

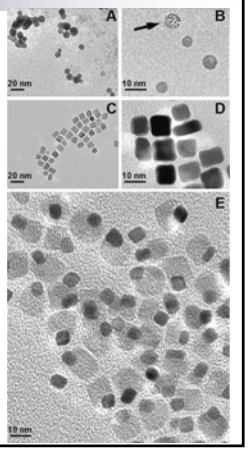
[Yan, Von Dem Bussche, Kane, Hurt, *CARBON*, in press]

- Many synthetic surfactants show appreciable toxicity and/or environmental health risk
- α-TocopherylPolyethyleneGlycolSuccinate (TPGS) is a water soluble form of vitamin E used as a dietary supplement and drug delivery vehicle
- TPGS cleaves by enzymatic hydrolysis to deliver the lipophilic α-tocopherol (Vitamin E) to cell membranes
- TPGS is commercially available. It is not marketed as a surfactant, but is an amphiphile based on structure
- Its interactions with nanotubes and fullerenes have never (to our knowledge) been studied



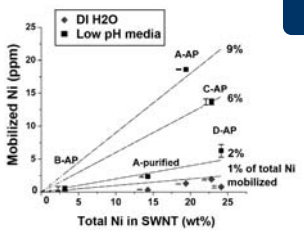
TPGS is an effective dispersant for MWNTs and shows a unique co-self-assembly with C60





TPGS is a more effective dispersant for MWNTs than Triton

4. Targeted removal of bioavailable metal as a nanotube detoxification strategy



The bioavailable metal represents only from 0.5% to 9% of the total metal

How can we reliably remove the *bioavailable fraction* of CNT metal?

Issues to address

A. What is the origin of bioavailable metal, especially in "purified" samples?

1. **kinetic limitations on acid washing?**
2. **surface re-deposition (ions, salts)?**
3. **oxidative carbon shell attack during or after acid wash?**

B. How can this bioavailable fraction be optimally removed without tube damage?

C. Will the non-bioavailable (encapsulated) metal be stable in the body? (a question of biopersistance of carbon shells)

Brief Summary

- All carbon nanotubes studied (as-produced and "purified") release free metal (Fe, Ni, Y) into physiological fluid phases, which trigger known toxicity pathways. Metal bioavailability is influenced by processing and environmental exposure. Metal bioavailability assays should be standard CNT characterization.
- Single-walled carbon nanotubes deplete essential micronutrients from medium by physisorption and can affect cell behavior by a new *indirect* mechanism.
- TPGS, a water-soluble Vitamin E formulation, is also a promising safe surfactant for carbon nanomaterial processing, esp. MWNT. Future work will attempt to use TPGS to actively mitigate oxidant damage associated with nanomaterial exposure.
- Bioavailable metal in nanotubes can likely be removed by selective targeting as a simple detoxification strategy (pending future work) .

BROWN

Acknowledgements

Financial support for work on nanotoxicology / safe nanomaterials:

- US EPA (STAR Grant RD83171901)
- NSF NIRT grant (DMI-050661)
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In the Kane Lab: Charles A. Vaslet, Ph.D., Annette Von Dem Bussche, Ph.D., Kevin McNeil, Michelle Buechner, Vanesa Sanchez, Jodie Pietruska, Ashley Smith

Environmental Impacts of Nanomaterials on Organisms and Ecosystems: Toxicity and Transport of Carbon-Based Nanomaterials across Lipid Membranes

This research is funded by U.S. EPA - Science To Achieve Results (STAR) Program
Grant # R832635

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Research Approach & Methods

Toxicology

– Screening NM using micro-biotests for potential toxicity

– Carbon-based, metal, and metal oxide NM and quantum dots

Biogeochemistry

– Effects on Ecosystem Functions

– Use toxic NM and a series of microbial driven reactions involved in sedimentary cycling of organic carbon to assess the potential impact of NM on basic ecosystem functions

Molecular Modeling & Microscopy

– Mechanisms of permeation of NM into the cell

– Assess possible damage to the cell membrane by NM

Microbiotests for Screening Studies

- Small-sized test species
- Rapid, simple, low-cost

Ceriodaphnia dubia Acute Toxicity Test

A short-term (48 hr) acute assay used to assess the toxicity of freshwater samples

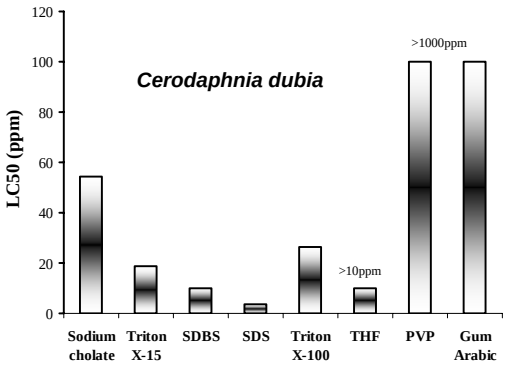
Selenastrum capricornutum Chronic Toxicity Test

May equal or even surpass that of the 48-hr Ceriodaphnia dubia acute testing

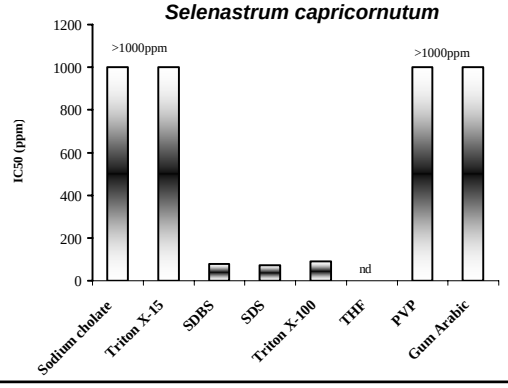
MetPLATE™

Based on the inhibition of the enzyme β-galactosidase by metals at toxic levels in a mutant strain of E. coli

Toxicity (LC_{50}) of Different Surfactants on Aquatic Organism Models



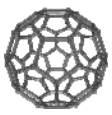
Toxicity (IC_{50}) of Different Surfactants on Aquatic Organism Models



C_{60} Toxicity

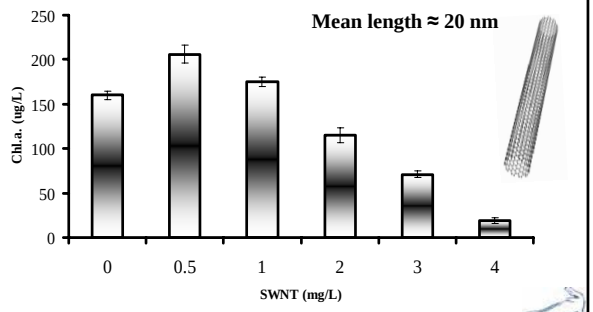
THF concentration < 0.1 ppm

Biotests	Observation	EC_{50} (ppm)
MetPlate	Not Toxic	-
Ceriodaphnia dubia	Toxic	0.43±0.11
Selenastrum capricornutum	Toxic	0.13±0.05



Effect of SWNTs on *S. capricornutum* 96-hour Growth Experiment

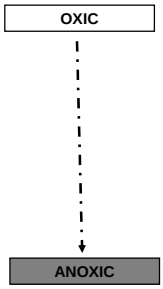
(Surfactant: 50 ppm Gum Arabic)



Biogeochemistry

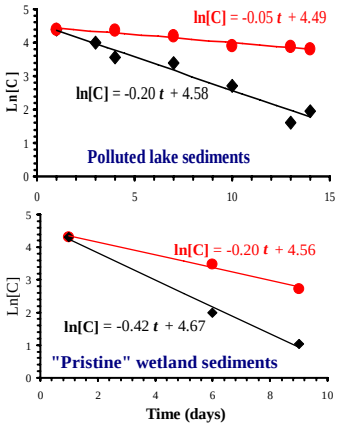
Assess Impacts of NM on Basic Ecological Functions

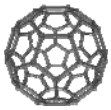
Organic Matter —(Ox/Red)→ $CO_2 + H_2O$



- Organic matter decomposition in sedimentary environments
- Microbial community structure and activity as indicator of ecosystem well being
- Measurement of predominant terminal electron-accepting processes in sediments

Microbial Degradation of Acetate in Sedimentary Environments





C_{60} concentration = 0.5 ppm

THF concentration < 0.1 ppm

♦ Control

● C_{60}- treated slurry

Preliminary Conclusions Toxicology & Biogeochemistry

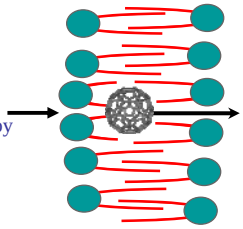
- **Microbiotests**
  - C_{60} and SWNT toxicity significantly exceeds solvent toxicity
- **Biogeochemistry**
  - C_{60} toxicity significantly exceeds solvent toxicity
  - Slows down metabolism of bacteria
  - Effect sensitive to soil composition
- **Open questions**
  - CWNT at small concentrations promote algae growth ?
  - Effect of trace metals in CWNT (MetPLATE™)
  - Fluorescence of CWNT
    - Transport into cell/cell membranes
    - Develop connection with molecuar modeling studies

Molecular Modeling

Task 1

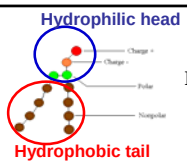
Understand Mechanism(s) of Permeation of NMs into Cell

- NM transport through cell membranes
- Model cell membrane as lipid bilayers
- NMs can penetrate cellular membranes by mechanism different from phagocytosis and endocytosis *(Rothen-Rutishauser et al., ES&T. 40, 4353, 2006)*
- Investigate effects of particle size and shape on transport



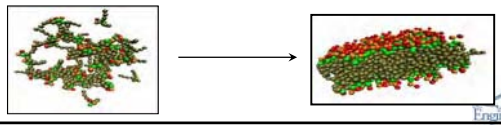
Coarse Grained Model

(*Marrink et al., J. Phys. Chem. B 2004, 108, 750*)



DPPC lipid

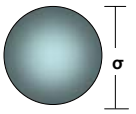
- Groups of atoms are mapped onto a single coarse-grained bead
  - *e.g., four CH_2 groups→ single hydrophobic bead*
- Good agreement with more detailed models
- Significant speed-up of simulations → fast screening of different nanoparticles
- Simulations with GROMACS MD package (www.gromacs.org)
- Bilayer preparation: self-assembly



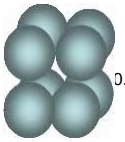
Coarse-Grained Nanoparticle Models

Fullerene-like particles

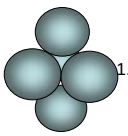
Spheres of diameters σ = 0.47 nm – 1.5 nm



Cube

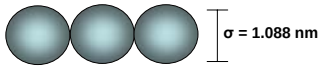


Tetrahedron



Rod-like particle

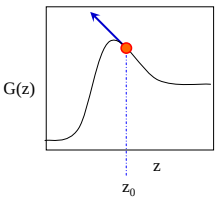
Chain of spherical particles



Calculation of Free Energy

Constraint Mean Force Method

Constraint force $F(z_0,t)$ to hold particle at $z = z_0$



$F(z_0,t)$, deterministic force:

$$< F > (z_0) = \frac{dG(z_0)}{dz}$$

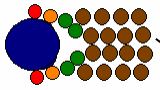
$F(z_0,t)$, instantaneous random force:

$$\Gamma(z,t) = F(z,t) - < F(z,t) > .$$

S. J. Marrink and H. J. C. Berendsen, J. Phys. Chem. ***98****, 4155 (1994)*

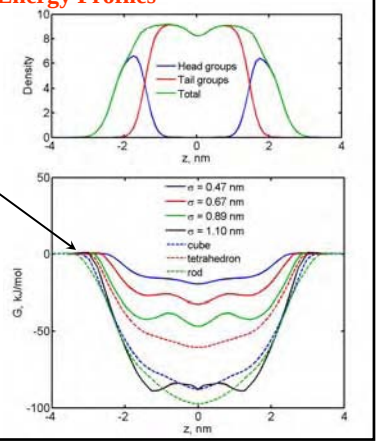
Free Energy Profiles

Negligible energy barrier for entry

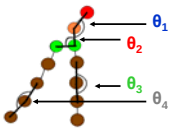


Significant energy well in bilayer center

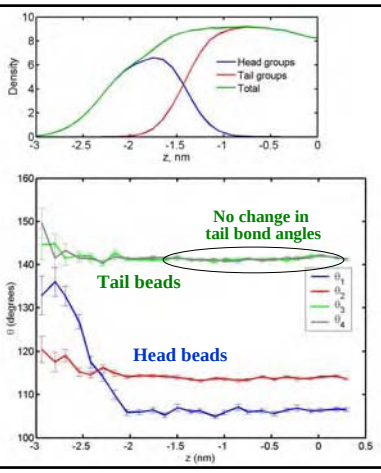
Qualitative differences between spherical and non-spherical particles



Local Lipid Structure



Lipid bond orientation in neighborhood of nanoparticle

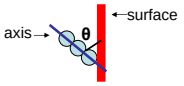


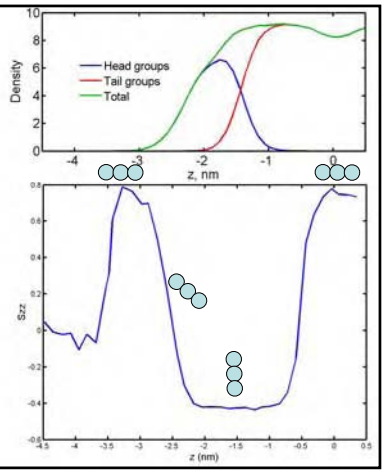
Rod-like Particle

Preferred orientation

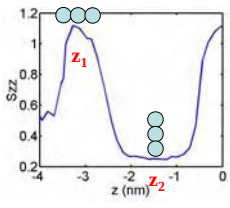
- S_{zz} = 1, perpendicular
- S_{zz} = -0.5, parallel
- S_{zz} = 0, no preference

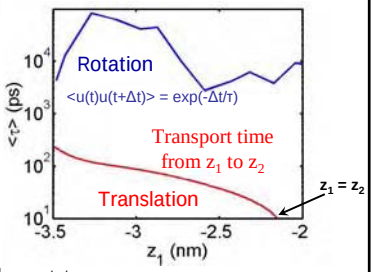
$$S_{zz} = \frac{1}{2}(\cos^2\theta - 1)$$





Rod-Like Particle: Translation & Rotation

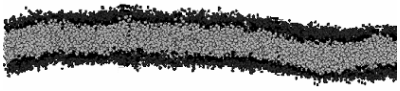




- Particles first move in and then rotate
- No permanent damage observed in constrained simulations
- Short-scale damage/pressure profile change?
- Constrained MD cannot predict dynamics correctly
- Use alternative approach: Kopelevich *et al.*, J. Chem. Phys.,2005

Task 2
Effects of Nanoparticles on Membrane Stability

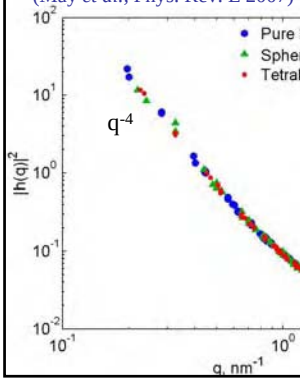
- Nanoparticles are observed to cause cytotoxicity by membrane rupture (e.g., *Sayes et al., Nano Letters, 10, 1881, 2004*)



- Focus on possible physical mechanisms
  - Bending modulus
    - Important for intracellular nutrient transport
  - Pressure profile
    - Affects membrane proteins
  - Disruption of equilibrium between lipid rafts

Preliminary Results

- Bending and tilt modulus from magnitude of bilayer fluctuations (May *et al.*, Phys. Rev. E 2007)



$$\left\langle |h(\mathbf{q})|^2 \right\rangle = k_B T \left(\frac{1}{k|\mathbf{q}|^4} + \frac{1}{k_\theta |\mathbf{q}|^2} \right)$$

k = bending modulus

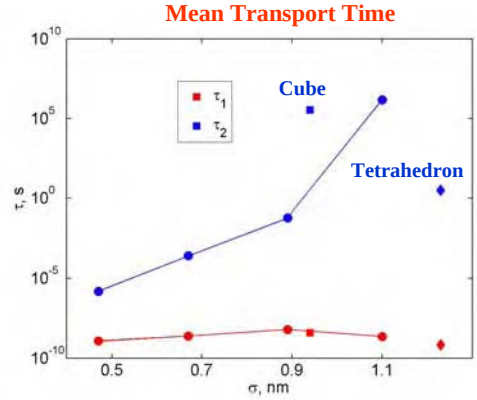
k_θ = tilt modulus

Membrane bending modulus is not affected

Summary
Molecular Modeling

- **No significant energy barrier to enter bilayer**
- **Long residence time inside bilayer**
- **Size and shape impact nanoparticle transport rates, dynamics, and localization within membrane**
- **Physical effects (preliminary data)**
  - Role of rotation of rod-like particles
  - Spherical and almost spherical particles:
    - No effect on bending modulus
- **Future work:**
  - Other physical effects, nanotube rotation
  - Effects of NM localization within cellular membrane

Mean Transport Time



τ_1 = time to enter membrane
τ_2 = time to enter cell interior

Assessment Methods for Nanoparticles in the Workplace

Patrick O'Shaughnessy

Department of Occupational and Environmental Health
College of Public Health
The University of Iowa

Overall Research Objectives

1. Identify and evaluate methods to measure airborne nanoparticle concentrations.
2. Characterize nanoparticles to assess their surface and bulk physical and chemical properties.
3. Determine the collection efficiency of commonly-used respirator filters when challenged with nanoparticles.

The University of Iowa

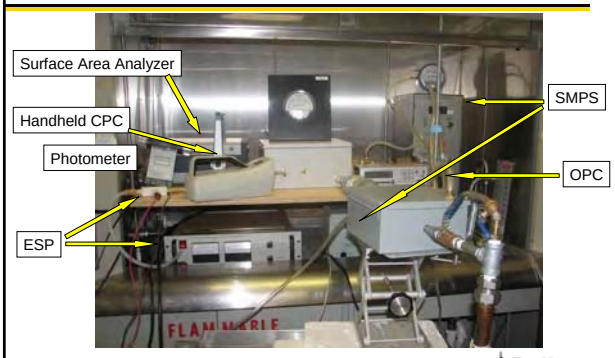
Instrument Comparison

Department of Occupational and Environmental Health
College of Public Health
The University of Iowa

Comparison Apparatus

The University of Iowa

Instruments Compared



The University of Iowa

Instrument Specifications

Instrument	Model	Application	Measured Unit	Limits	Particle Size Range, nm
TSI Handheld CPC	3007	Count	#/cm^3	0 - 10^5	10 -1000
TSI CPC	3010	Count	#/cm^3	10^{-4} - 10^4	10 – 3000
TSI DMA	3071	Count/Diam	#/cm^3	NA	5 – 1000
GRIMM OPC	1.108	Count	#/1000 cm^3	0 – 2x10^6	300 – 20,000
Matter Inst. SAA	LQ1-DC	Surface Area	μm^2/cm^3	0 - 2000	10 - 80

The University of Iowa

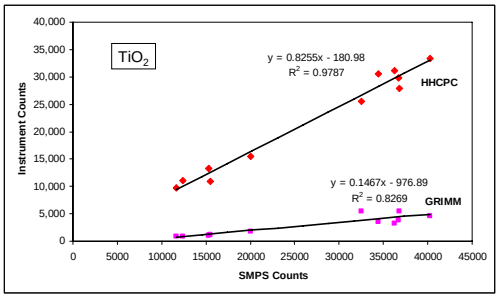
Powder Types Analyzed

- Iron Oxides:
  - High Concentration
  - Medium Concentration
- Titanium Dioxides
  - High Concentration
  - Medium Concentration
  - Low Concentration
- Single Walled Carbon Nanotubes

TiO_2 Comparison

Trial Sets	Geometric Mean (nm)	GSD	SMPS Average Count (#/cm³)	GRIMM Average Count (#/cm³)	CPC Average Count (#/cm³)	LQ1 (μm²/cm³)	Average SMPS Surface Area (μm²/cm³)
AVG I	89.2	2.5	13,120	915	11,363	264	930
AVG II	118.3	2.1	17,735	1,500	13,197	450	1,782
AVG III	129.1	1.9	35,571	3,754	30,197	1,205	3,171
AVG IV	151.4	1.6	34,624	5,480	26,696	1,556	3,548
AVG V	104.7	2.4	38,252	3,925	32,282	604	3,157

Count Correlations



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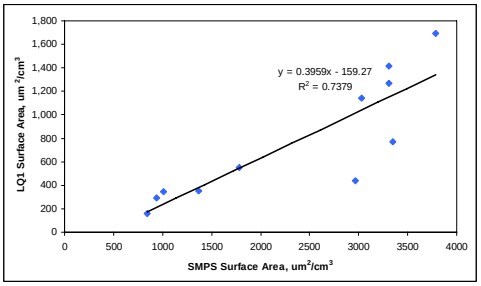
Surface Area Correlations



Aerosol Generation

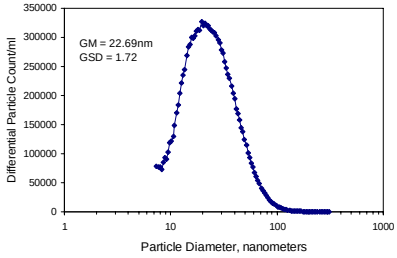
Collison Nebulizer

Added bulk powder to filtered water
Nebulized at 20 psi

Instrument Comparison

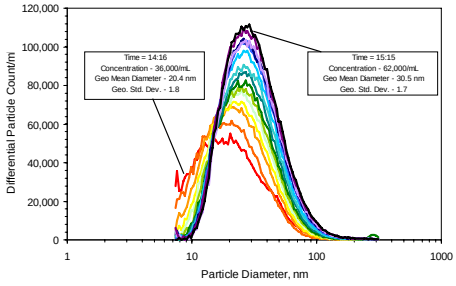
Water Contamination

Typical Water Only Results

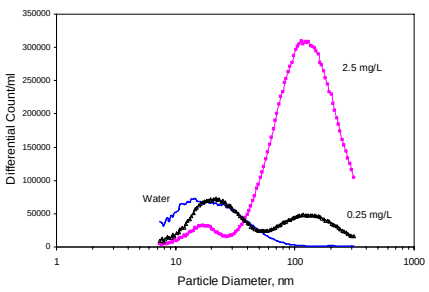


6-jet Collison Nebulizer
Ultrapure Water from Lab System

Water Output over Time

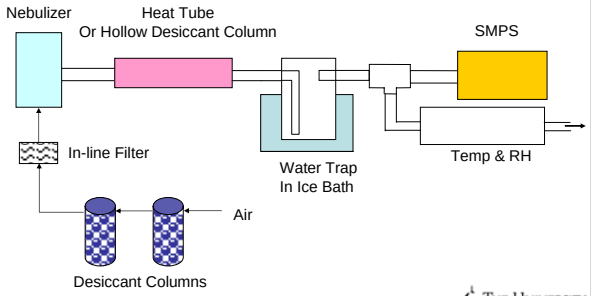


Nebulizer Output with Powder

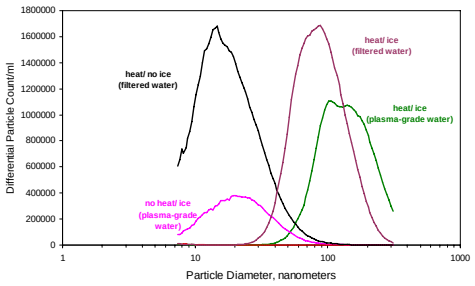


6-jet Collison Nebulizer
Ultrapure Water from Lab System
20-nm TiO2 Added and ultra-sonicated

Water Trials



Water Trials



Park Work\SMPS Files\Dec 15 Water Setup Tests\comparison

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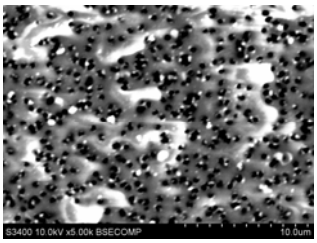
SEM of Water



Park Work\SEM-EDS Jonas Results\PC membrane_filtration

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SEM-EDS

Spectrum: 2hr_Cfilter 1

El	AN	Series	unn. C [wt.-%]	norm. C [wt.-%]	Atom. C [at.-%]	Error [%]
N	7	K-series	21.95	21.95	30.31	9.0
Cu	29	L-series	19.79	19.79	6.02	8.2
Na	11	K-series	5.54	5.54	4.66	0.5
Cl	17	K-series	2.39	2.39	1.31	0.1
P	15	K-series	1.97	1.97	1.23	0.1
K	19	K-series	1.37	1.37	0.68	0.1
Mg	12	K-series	1.34	1.34	1.07	0.1
Si	14	K-series	0.88	0.88	0.61	0.1
O	8	K-series	44.77	44.77	54.12	15.2
		Total:	100.00	100.00	100.00	

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Instrument Issues

Department of Occupational and Environmental Health
College of Public Health

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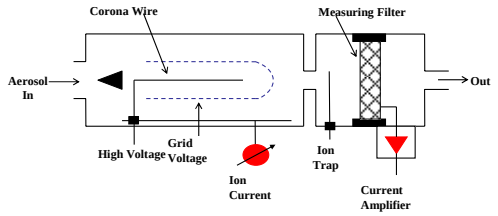
Surface Area Analyzer



- Particles are charged by unipolar diffusion of ions from the corona charger.
- A filter downstream from the charger measures the current of the particles via an electrometer.
- Active surface area (not individual particle surface area) is calculated from the measured current.

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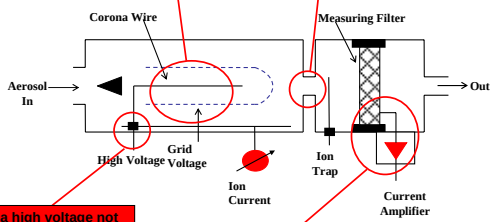
Areas of Degradation

Deposition of material on wire increases wire diameter. Voltage increases and eventually results in an incorrect display reading. Replaced part.

Connection between charger and filter became brittle. Replaced part.



Corona high voltage not constant due to a defective electronic part. Causes wrong or instable display reading. Replaced part.

Measuring filter – lack of sensitivity. Caused by deposition of material in the glass/metal feedthrough and changing humidity. Replaced part.

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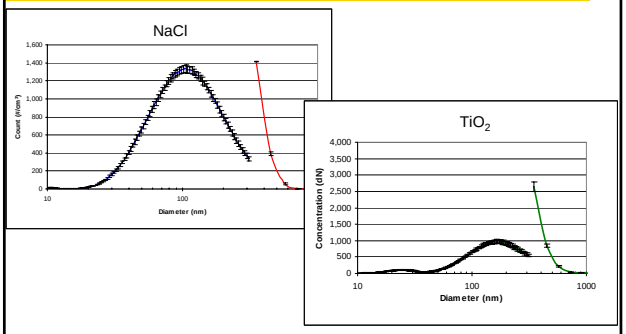
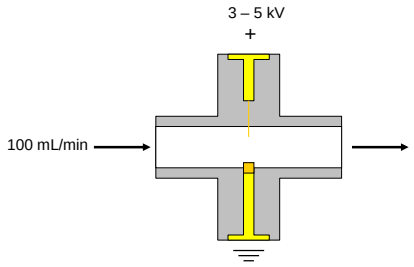
SMPS and GRIMM Size Distributions



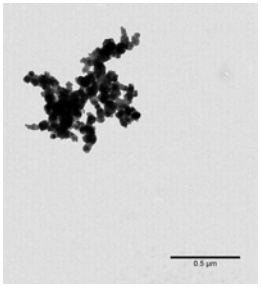
Microscopic Sizing

ESP

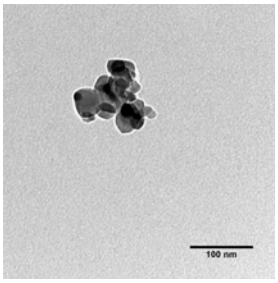


TEM Imaging/Counting

Large Fe_2O_3 Agglomerate

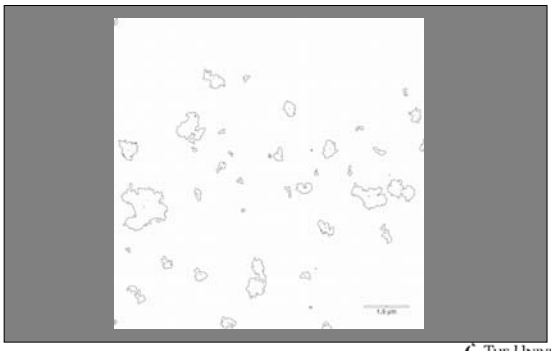


Small TiO_2 Agglomerate



Captured on TEM grid via ESP collector

TEM Imaging/Counting



Park Work\TEM Images\Summary\Fe2O3\..s files

Particle Characterization

Characterization Techniques

Technique	Information
XPS	Elemental Composition
XRD*	Crystallinity
SEM TEM	Shape, homogeneity, tube size, size distribution, and surface morphology
BET	Surface area
Raman spectroscopy	Tube diameter, conductivity, purity
AFM*	Tube length, diameter

* Future work

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TiO_2 Analysis

Crystalline or Amorphous	Crystalline
Phase	Anatase
Primary Particle Diameter (nm)	4 ± 1
BET Surface Area (m^2/g)	266 ± 3
Surface Functionalization	O, O-H, and H_2O
Aerosol Aggregate Size	100 ± 50 nm

The University of Iowa

Carbon Nanotube Analysis

	SWNT	DWNT
Average Diameter (nm)	4.5 ± 3	2.8 ± 2
Surface Area (m^2/g)	457 ± 4	575 ± 10
Catalyst Contamination	Co < 0.2%	Not detectable
Conductivity	Semiconducting	Semiconducting

The University of Iowa

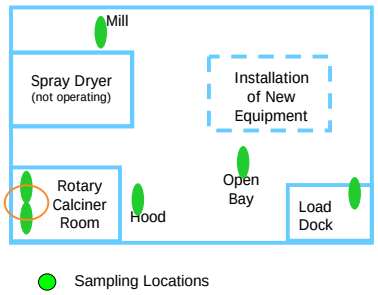
Field Sampling

Nano-structured Lithium Titanate Facility

Department of Occupational and Environmental Health
College of Public Health

The University of Iowa

Facility Schematic



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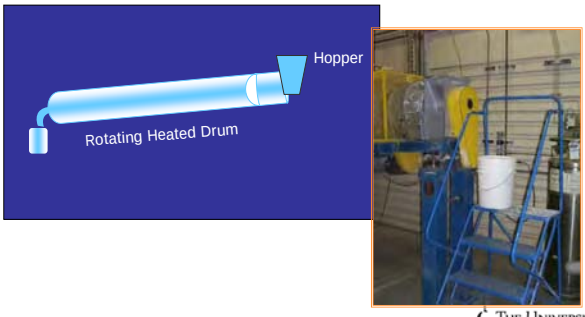
Rotary Calciner



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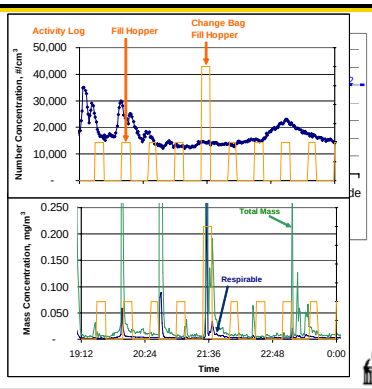
Real-time Measurements



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Conclusions

- Material handling of lithium titanate disperses large particles (>1 μm)
- Ultrafine particles likely associated with forklifts, welding, grinding

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Acknowledgements

Faculty
- Thomas Peters
  - Field Assessments
- William Heitbrink
  - Filtration Expertise
- Vicki Grassian
  - Particle Characterization

Students
- Linda Schmoll – PhD
  - Instrument Comparison
  - Filtration Studies
- Sherrie Elzey - PhD
  - Particle Characterization
- Hyun Ju Park – MS
  - Water Contamination & TEM/SEM
- Ron Johnson – MS
  - Field Sampling

Staff
- Jonas Baltrusaitis
  - SEM & TEM Analysis

Funding

NIOSH National Institute for Occupational Safety and Health

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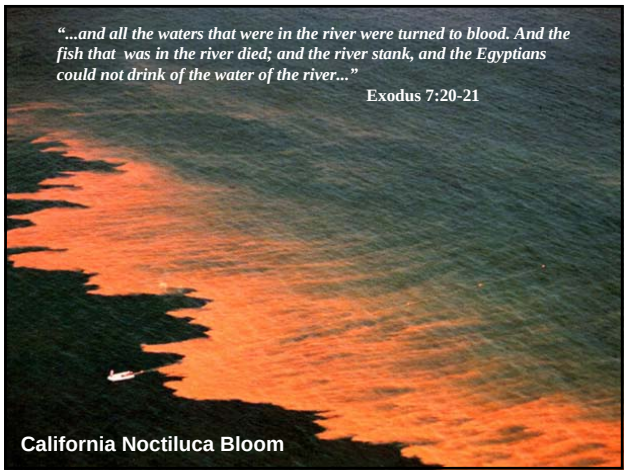
Protecting the environment from nanotechnology

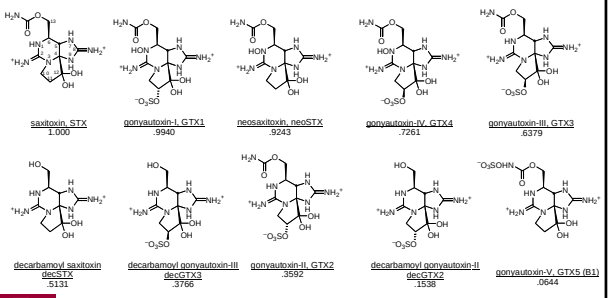
Robert Gawley

Department of Chemistry and Biochemistry

University of Arkansas, Fayetteville, Arkansas



Paralytic Shellfish Toxins



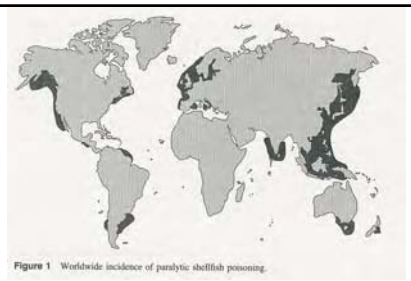
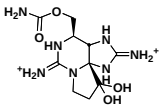
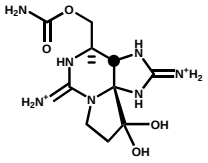


Figure 1 Worldwide incidence of paralytic shellfish poisoning.



saxitoxin, STX

- Vector: shellfish
- Saxitoxin (and tetrodotoxin) bind to orphan site 1 on voltage-gated sodium channels



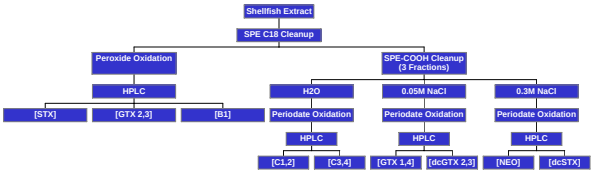
Saxitoxin, STX

Benchmark method for detection: Mouse Bioassay

- Mush up 15-20 clams
- Boil 5 min in 0.1N HCl
- Adjust to pH 3
- Inject into mice, time 'til death

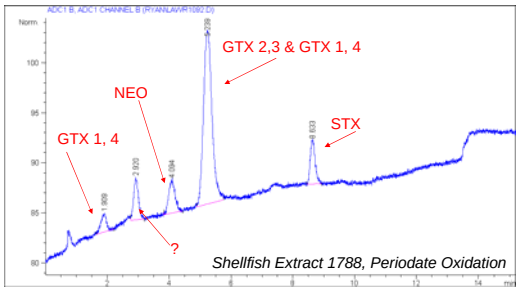
Legal limit: 80 μg/100 g shellfish
Detection limit: 40 μg/100 g; ~ 1 μM

The Lawrence Method



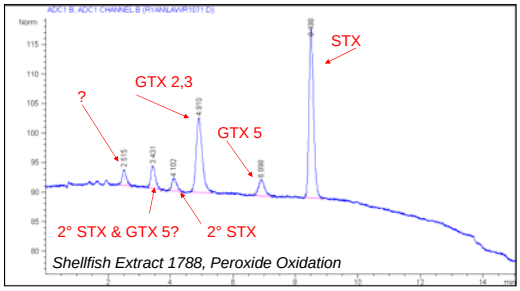
Periodate Oxidation of Shellfish Extract

- Shellfish Extract 1788 was oxidized with periodate and injected into the HPLC with a 50µL sample loop.



Peroxide Oxidation of Shellfish Extract

- Shellfish Extract 1788 was oxidized first by peroxide and injected into the HPLC with a 10µL sample loop



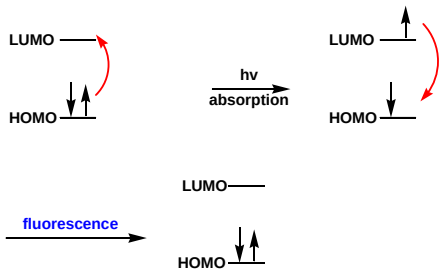
Fluorescence Sensing

$$STX + Sensor \underset{}{\overset{K_{11}}{\rightleftharpoons}} STX{\bullet}Sensor$$

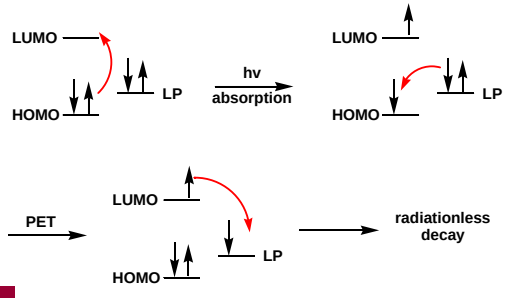
$$K_{11} = \frac{[STX{\bullet}Sensor]}{[STX][Sensor]}$$

Fluorescent

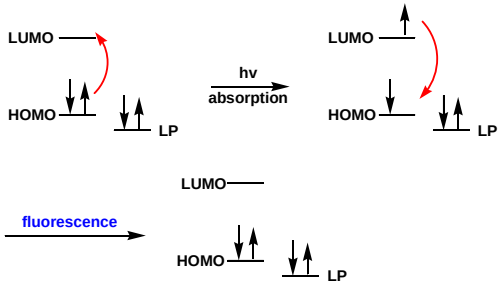
Fluorescence



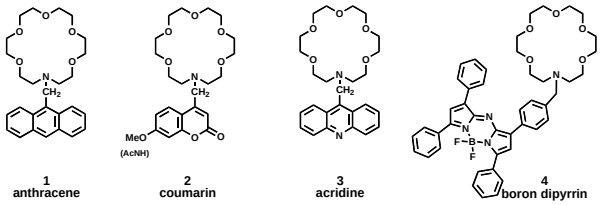
Photoinduced Electron Transfer



Complexed Host



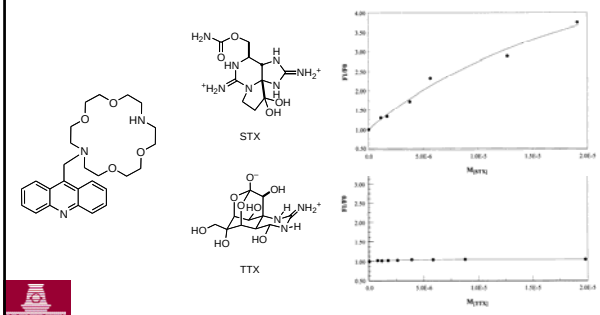
Host Fluorophores



1	2	3	4
anthracene	coumarin	acridine	boron dipyrrin
366,390 / 420	328 / 420	350 / 420	644 / 677

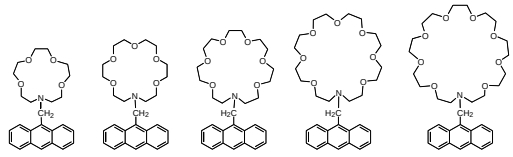
λ_{max} absorption / emission

Selectivity: STX *vs* TTX



Toxicon **2005**, *45*, 783; corrigendum *46*, 477

Effect of Crown Size



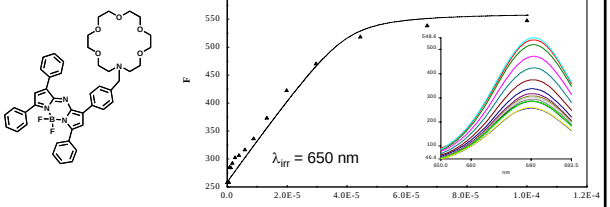
15-crown-5	18-crown-6	21-crown-7	24-crown-8	27-crown-9
4.93	5.3	4.68	10.7	16.9

$K_{11} \times 10^4\ M^{-1}$

Hua Mao

Can. J. Chem., **2006**, *84*, 1273

BDP Crown Binding Isotherm

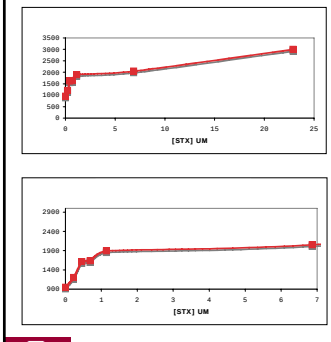


K = 2.9 - 8.9x10^5 M^{-1} in methanol

Jack Thorne
Jennifer Pharr

J. Org. Chem. **2007**, *72*, 2187

New Class of Crowns



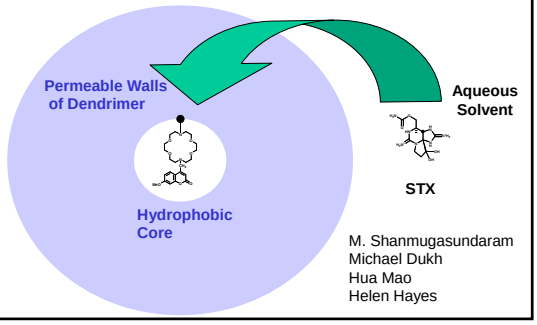
[crown] = 20 µM

Expansion of top trace

100% Fluorescence Enhancement at 1 µM [Saxitoxin]

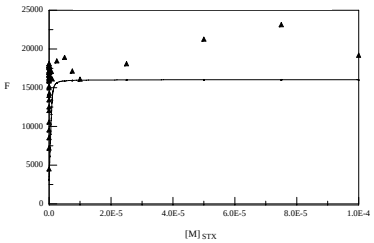
Hua Mao
Jack Thorne

Coumarin Sensor Anchored in Nanoenvironment of Dendrimer



M. Shanmugasundaram
Michael Dukh
Hua Mao
Helen Hayes

Binding Isotherm (Coumarin Fluorophore)



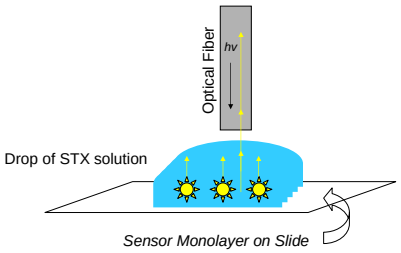
$K_{11} = 1.2 \times 10^8\ M^{-1}$

M. Shanmugasundaram
Jack Thorne

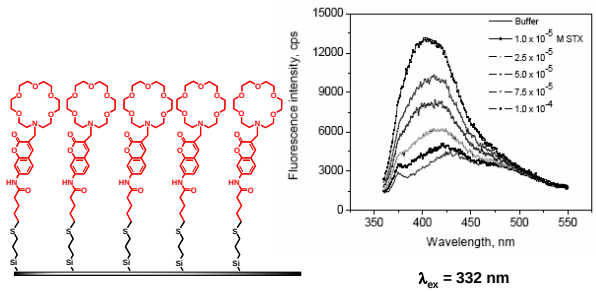
Factors Influencing Enhancement

- Polarity of environment
- Solvation (entropy)
- Ion Pairing
- Dilution artifacts?

Sensing in a Nanoscale SAM



Sensor on a Monolayer



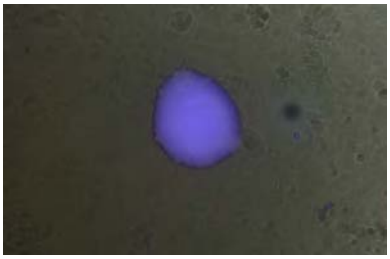
λ_{ex} = 332 nm

Chem Commun **2006** 1494

(with Leblanc group)

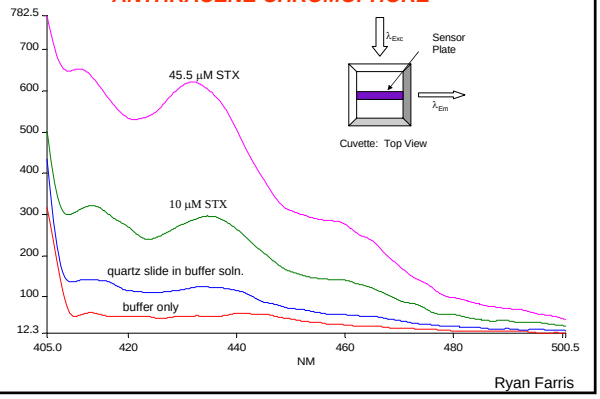
Drop of 0.1 M acetic acid on chemosensor modified quartz

Anthracene chromophore



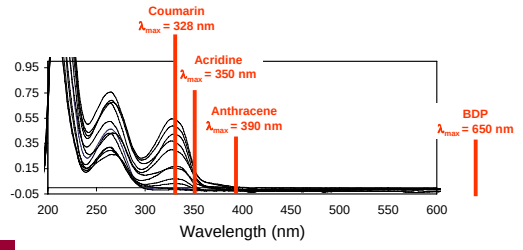
Emission Spectra of Modified Quartz at Various Saxitoxin Concentrations

ANTHRACENE CHROMOPHORE



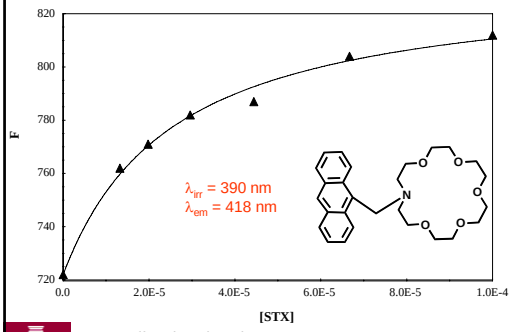
Ryan Farris

Shellfish Extracts: Absorption



Jack Thorne
Jennifer Pharr

Spiked Nontoxic (preliminary)

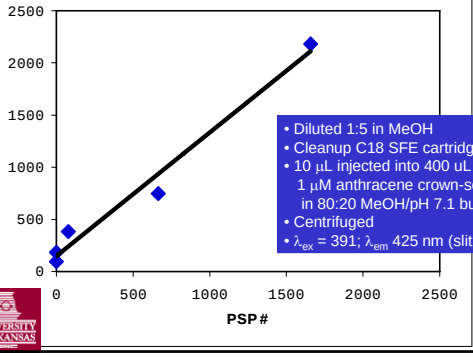


Buffered methanol:
pH 7.1 (tetrabutyl ammonium phosphate

Jack Thorne
Jennifer Pharr

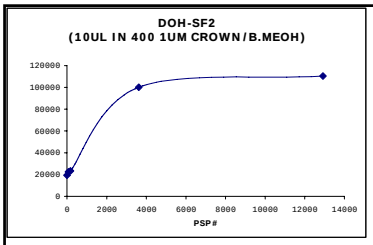
DOH Shellfish Extracts

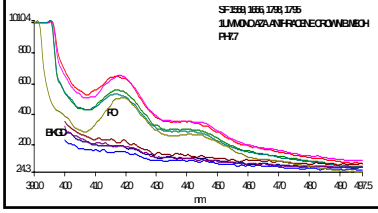
2005 *Alexandrium* bloom, Seattle



- Diluted 1:5 in MeOH
- Cleanup C18 SFE cartridge
- 10 μL injected into 400 uL of 1 μM anthracene crown-sensor in 80:20 MeOH/pH 7.1 buffer
- Centrifuged
- λ_{ex} = 391; λ_{em} 425 nm (slit 5x5 nm)

Jack Thorne
Jennifer Pharr



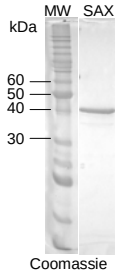


2006 *Alexandrium* bloom

Extract of blue mussels

Bob Lona
Jack Thorne

Saxiphilin & C-Lobe

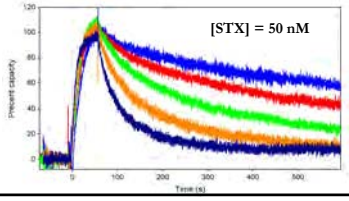


With Henry and Fritsch groups

http://www.exploratorium.edu/frogs/mainstory/frogstory2.html

Kinetics Measurements

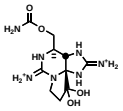
Temp	k_a x 10^6 ($M^{-1}s^{-1}$)	k_d x 10^{-3} (s^{-1})	K_D (nM)
11	1.108 ± 2	0.980 ± 3	0.884 ± 3
17	1.83 ± 1	2.1 ± 1	1.153 ± 4
25	2.675 ± 9	4.1 ± 1	1.536 ± 5
31	2.68 ± 1	8.35 ± 4	3.12 ± 1
37	5.47 ± 7	27.2 ± 3	4.98 ± 2



Penny Lewis

Thermodynamics

- **Δ G° = –12,000 cal/mol**
  - Binding spontaneous, exergonic
- **Δ H° = –11,650 cal/mol, Δ S° = 350 cal•mol^{-1}•K^{-1}**
  - Binding exothermic
  - Entropy small—most binding energy from enthalpy
  - ΔC_p = –720 cal/mol·K
  - *Probably involves burying of hydrophobic surfaces*
  - *Possibly some burial of water molecules*



Penny Lewis

Thanks to:

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Ms Penny Lewis
Mr Ryan Farris
Prof Ralph Henry
Prof Ingrid Fritsch
Prof Roger Leblanc (U Miami)
Prof Rick Tarkka (UCA)

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Washington DOH: Dr Bob Lona (Shellfish)

Evaluating the Impacts of Nanomanufacturing via Thermodynamic and Life Cycle Analysis

Bhavik R. Bakshi and L. James Lee
Vikas Khanna, Geoffrey F. Grubb, Yi Zhang

Department of Chemical and Biomolecular Engineering
The Ohio State University, Columbus, Ohio, USA

Interagency Workshop on the Environmental Implications of Nanotechnology

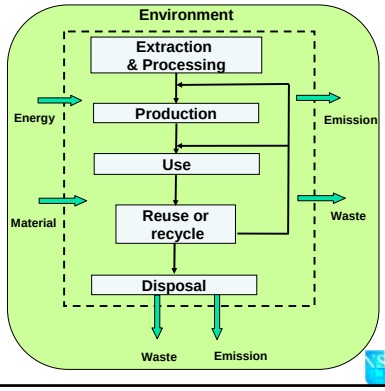
September 5-7, 2007 Washington, DC

Motivation

- Discover problems with technology *before* it is fully developed and adopted
- Guide development of nanotechnology to be environmentally benign and sustainable
- Understanding environmental impact of nanomaterials is essential but not enough
- Need to adopt a *systems view* with *life cycle thinking*
- Life Cycle Analysis of emerging technologies poses unique challenges

Life Cycle Analysis

- Need data for each stage of life cycle
  - Energy
  - Materials
  - Emissions
  - Impact
- Difficult to find for emerging technologies



Challenges in LCA of Nanotechnology

- Inventory for nanomanufacturing is not available
- Impact of engineered nanomaterials on humans and ecosystems is only partially known
- Predicting life cycle processes and activities is difficult since the technology is still in its infancy

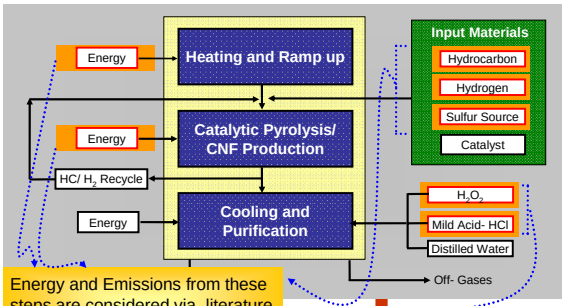
Objectives

- Life Cycle Evaluation of Nanoproducts & Processes
  - Establish Life Cycle Inventory modules for Nanomaterials
  - Perform LCA of Polymer Nanocomposites Products
- Explore predictive model for LCA and impact assessment
  - Relationship between life cycle inputs and impact
  - Relationship between properties of nanoparticles and their impact

LCA of Carbon Nanofibers

- Extraordinary high tensile strength
  - Tensile strength-12000 MPa, *10 times that of Steel*
  - Increases mechanical and impact strength of polyolefins
- Near term applications in Polymer Nanocomposites
  - ***Automotive Body Panels***
  - Expected to replace Steel and Aluminum
- CNFs show more commercial potential compared to Carbon Nanotubes (CNTs)

System Boundary



NANOFIBERS

Khanna, V., Bakshi, B. R., Lee, L. J., 2007

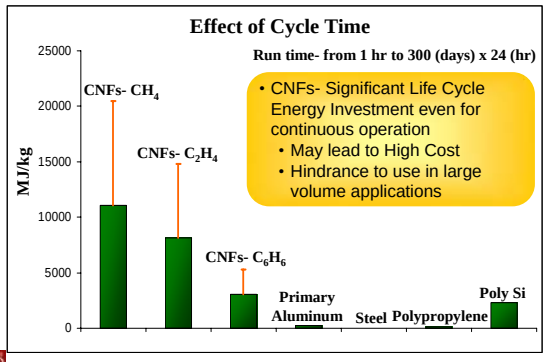
Data Sources and Assumptions

- Process data
  - Journal papers and conference books
  - Encyclopedias of chemical engineering and chemistry
- Inventory data
  - SimaPro, (PRé Consultants); NREL
- Impact assessment
  - CML-IA (Leiden Univ. Institute of Environ. Sci.)
  - Eco-Indicator 99

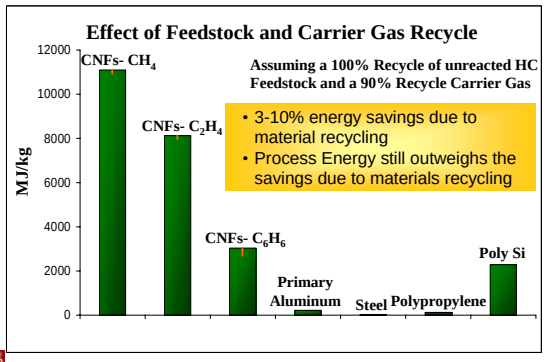
Assumptions

- Impact of Nanoparticles is not accounted
- Purification efficiency of 90%
- Catalyst life cycle is ignored (BEST Case)
- 100% efficiency for electric heating (BEST case)

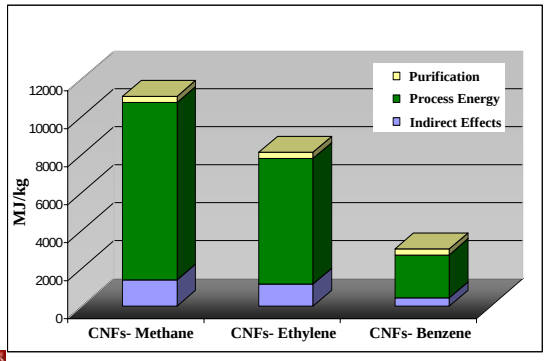
Results-Life Cycle Energy Analysis



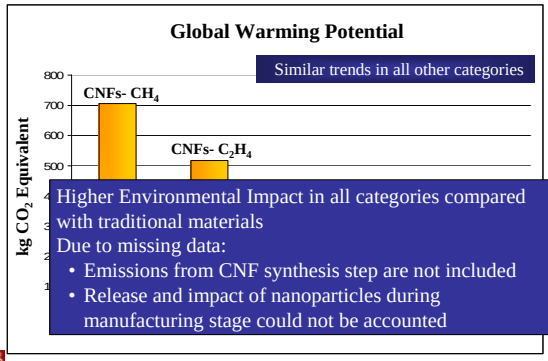
Life Cycle Energy and Process Parameters



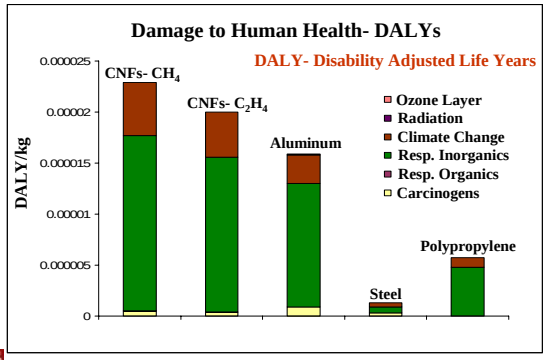
Life Cycle Energy Analysis- Cont'd



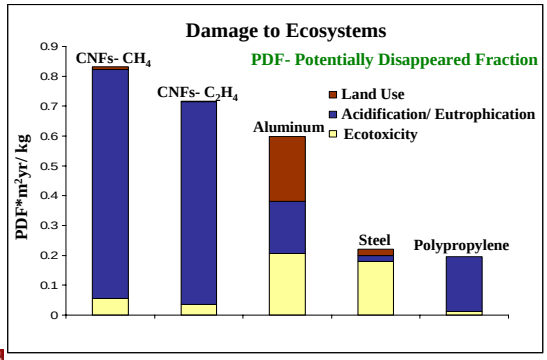
Impact Assessment- Midpoint Indicators



Impact Assessment-Damage Indicators



Impact Assessment-Damage Indicators



Conclusions for First Objective

- On an equal mass basis:
  - CNFs require significantly higher energy investment compared with traditional basic materials
  - CNFs do seem to have a larger life cycle environmental impact than traditional materials
- High energy may lead to high cost thus restricting use of CNFs only in *niche applications*
- Products based on Carbon Nanofibers may be greener than alternatives for certain applications
  - Quantity will be the deciding factor

Objectives

- Life Cycle Evaluation of Nanoproducts & Processes
  - Establish Life Cycle Inventory modules for Nanomaterials
  - Perform LCA of Polymer Nanocomposites Products
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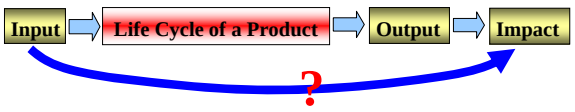
  - Relationship between life cycle inputs and impact
  - Relationship between properties of nanoparticles and their impact

LC Impact and Input-side Analysis



- LCA is primarily an *output-side* method, but *input-side* information is more readily available
- Can *input-side* information provide an indication of life cycle impact?

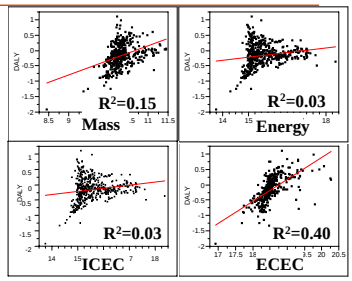
Zhang, Y., Bakshi, B. R., *IEEE Symposium on Electronics and the Environment*, 2007

Approach

- Identify relationship between inputs and impacts based on LCA of common products and processes
- Extract empirical model via rigorous statistical methods
- Aggregation of inputs is crucial
  - Mass
    - Ignores some types of energetic inputs
  - Energy
    - Ignores non fuel inputs
  - Exergy
    - Captures ability to do work, accounts for material and energy inputs
    - With and without work done by ecosystems
- If a relationship is found, it can be used for predictive LCA of emerging technologies

Preliminary Results

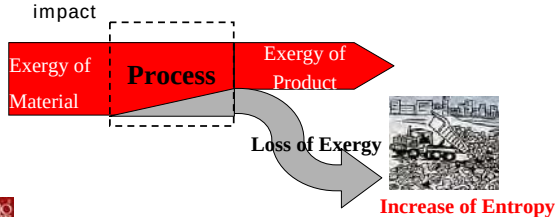
- Modeled relationship between mass, energy, ICEC, ECEC and human impact of emissions
- Based on thermodynamic model of U.S. economy*
- ECEC provides best fit
- Similar results via other studies
- Need to do more studies



* Ukidwe, N. U., Bakshi, B. R., *Energy*, 2007

Second Law and Environmental Impact

- Exergy is not conserved, it can be lost
- Manufacturing involves reduction of product entropy
- This results in an increase of entropy in the surroundings, which comes from the loss of exergy
- For same functionality, more exergy loss should mean more increase in entropy of surroundings and larger impact



Conclusions for Second Objective

- For emerging technologies, input information is easier to obtain
- Preliminary studies indicate promising correlation between life cycle inputs and impact
- Ecological cumulative exergy consumption seems best for aggregating inputs for Predictive LCA
- Relationship between toxicology of nanoparticles and thermodynamic properties is also promising
- More work is needed

Future Work

- LCA of conventional versus nanocomposite materials
- Further statistical evaluation of relationship between inputs and impact
- Explore relationship between thermodynamic properties of nanoparticles and their toxicity
- Risk analysis

- Acknowledgements
  - Financial support from EPA (Grant No. R832532) and NSF NSEC at Ohio State

U.S. Environmental Protection Agency
Interagency Workshop on the Environmental Implications of Nanotechnology

Hotel Monaco
Washington, DC

September 5–7, 2007

EXECUTIVE SUMMARY

SEPTEMBER 5, 2007

INTRODUCTION AND OVERVIEW

The 2007 Interagency Workshop on the Environmental Implications of Nanotechnology was held September 5–7, 2007, in Washington, DC, and was hosted by the U.S. Environmental Protection Agency (EPA), Office of Research and Development (ORD), National Center for Environmental Research (NCER). The workshop brought together research grantees funded by the EPA Science To Achieve Results (STAR) Program, the National Science Foundation (NSF), the National Institute of Environmental Health Sciences (NIEHS), and the National Institute for Occupational Safety and Health (NIOSH). Grantees discussed the latest science regarding the potential effects of engineered nanomaterials on human health and the environment. Additional talks were given by federal agency program officials. The goal of the workshop was to stimulate communication and collaboration among scientists and engineers investigating the potential implications of engineered nanomaterials.

Welcome and Introduction
Gary Foley, U.S. EPA

Dr. Foley welcomed participants and remarked that this workshop would provide an opportunity to examine the progress achieved by all research funding programs represented at the workshop. This effort is a partnership among agencies, in which the Department of Energy (DOE), NSF, NIOSH, NIEHS, and EPA work together. This coordination, one of many that have been initiated through involvement in the National Nanotechnology Initiative (NNI), seeks to assess implications and applications of nanotechnology. EPA currently is evaluating nanotechnology research needs across the Agency to determine its next steps with respect to nanotechnology research. NCER administers EPA's extramural research, including research grants and cooperative agreements, the fellowship program, and the Small Business Innovation Research (SBIR) program. Each of these programs includes a nanotechnology topic for proposal submissions. Overall, across all environmental topic areas, NCER makes about 300 awards each year. NCER typically administers approximately 1,000 active grants. The investigator-initiated research funded by these programs over the past 5–6 years is helping to pave the way for EPA's intramural research program to examine nanotechnology applications relevant to the Agency's mission.

The Agency is developing a Nanomaterial Research Strategy (NRS). The scope of this research document discusses broad themes and general approaches for extramural and in-house nanotechnology research. ORD has identified four key research themes and seven key scientific questions addressing the research themes where we can provide leadership for the federal government research program and support the science needs of the Agency:

(1) Sources, Fate, Transport, and Exposure

- Which nanomaterials have a high potential for release from a life cycle perspective?
- What technologies exist, can be modified, or must be developed to detect and quantify engineered materials in environmental media and biological samples?
- What are the major processes or properties that govern the environmental fate of engineered nanomaterials, and how are these related to the physical and chemical properties of those materials?
- What are the exposures that will result from releases of engineered nanomaterials?

(2) Human Health and Ecological Research To Inform Risk Assessment and Test Methods

- What are the effects of engineered nanomaterials and their applications on human and ecological receptors, and how can those effects be best quantified and predicted?

(3) Risk Assessment Methods and Case Studies

- How do Agency risk assessment and regulatory approaches need to be amended to incorporate the special characteristics of engineered nanomaterials?

(4) Preventing and Mitigating Risks

- What technologies or practices can be applied to minimize risks of engineered nanomaterials throughout their life cycle, and to apply nanotechnology to minimize risks posed by other contaminants?

The purpose of the NRS is to guide the ORD program in nanomaterial research. Anticipated outcomes from this research program will be focused research products to address risk assessment and management needs for nanomaterials in support of the various environmental statutes for which EPA is responsible.

PROGRAM PRESENTATIONS

How the National Nanotechnology Initiative is Addressing Environmental, Health, and Safety Research Needs

Celia Merzbacher, Office of Science and Technology Policy (OSTP), Executive Office of the President

NNI is the multi-agency program that coordinates all federal nanoscale research and development activities. The annual NNI supplement to the President's Budget reports investment in a number of areas including environmental, health, and safety (EHS) research. The primary purpose of research and development reported in this category is "to understand and address potential risks to health in the environment posed by nanotechnology." The NNI has reported on expenditures for EHS research and development by all agencies participating in NNI for each year since 2006. The amount being invested in EHS research grew from $37.7 million in 2006 to a request for $58.6 million in 2008—an increase of about 55%. Eight agencies plan to invest in EHS research in 2008. The NNI plans to spend $1.5 billion in 2008 on all aspects of nanotechnology research, including about $300 million on the nanomaterials category. Examples of EHS research include: (1) U.S. Department of Agriculture-funded research on reactivity, aggregation, and transport of nanocrystalline oxides in soil; (2) a U.S. Air Force-funded

multidisciplinary university research initiative to study the relationship between physical and chemical characteristics and toxicological properties of nanomaterials; and (3) NIOSH-funded research to develop verified instruments and methods for accurately assessing airborne concentrations of nanoparticles and the efficacy of respirator use for controlling exposure. EHS research, as defined by NNI for purposes of budget reporting, does not include critical research that has other primary purposes. For example, the National Cancer Institute (NCI) is funding a project on functionalized nanomaterials for cancer detection and treatment. In addition, the National Institute of Standards and Technology (NIST) is developing methods for the chemical characterization of nanoscale materials in three dimensions.

The Nanotechnology Environmental and Health Implications (NEHI) Working Group is a subgroup of the National Science and Technology Council, Nanoscale Science, Engineering, and Technology Subcommittee. The NEHI Working Group is co-chaired by the Food and Drug Administration and EPA's ORD; its 19 member agencies include both research and regulatory agencies. The NEHI Working Group provides an opportunity for information exchange and aims to identify and address EHS research needed to support regulatory decisionmaking. Five high-level categories of EHS research, identified by the NEHI Working Group in the report, *Environmental, Health, and Safety Research Needs for Engineered Nanoscale Materials*, include: (1) instrumentation, metrology, and analytical methods; (2) nanomaterials and human health; (3) nanomaterials and the environment; (4) health and environmental exposure assessment; and (5) risk management methods. These research needs were prioritized according to three overarching principles. First, NEHI prioritized research to maximize the value of information to be gained, such as the extent to which the research findings would reduce uncertainty, how broadly applicable the information would be, and the expected level of exposure. Second, NEHI sought to leverage investments by other stakeholders, such as industry and other countries. Third, to maintain awareness of the state-of-the-art, NEHI will periodically reassess these priorities. The research priorities were released for public comment in August 2007; the interim document is available at http://www.nano.gov. NNI will compare these priorities with current research to identify any gaps and areas of overlap and will develop a research strategy to address unmet research needs.

Those who are managing and performing research to address EHS issues related to nanotechnology should keep in mind the following additional points:

- Other stakeholders, in addition to the federal government, have a role in developing information about the potential risks of nanomaterials, including manufacturers and other countries.

- In addition to understanding the absolute effects of nanomaterials, it also is important to understand net risks. For example, the risks of certain materials or technologies may be acceptable if they are replacing more harmful alternatives.

- Research to understand implications should be integrated with basic and application-oriented research, both at the level of funding agencies and at the level of the individual researcher.

- Exposure must be studied in addition to toxicity.

- Further standards (e.g., standard reference materials and standard methods) are needed to ensure that nanotechnology research findings are comparable across countries.

- It is important to understand public perception of the risks and benefits of nanotechnology, as well as to communicate risk information that is useful to the public.

NNI and nanotechnology research are priorities of the administration. The OSTP's priorities memo for FY 2009, which is now available at the OSTP Web Site, includes a list of priority areas, including EHS

research related to nanotechnology. Thus, at the highest levels, the administration is directing agencies to implement the research needs that they have identified. The work of the researchers at this meeting is vital to the success of NNI.

Discussion

A participant asked Dr. Merzbacher why information on the budget for EHS research begins in 2006, rather than in 2001, when NNI was established. Dr. Merzbacher explained that the Office of Management and Budget did not begin asking NNI to break down budget information into different categories until 2006. Prior to that point, NNI collected this information more broadly, without separating it into categories. The participant also asked why NNI spent less than 4% on EHS implications of nanotechnology. Dr. Merzbacher said that the agencies are investing in research areas other than implications. She suggested that it is not a question of the percentage spent on EHS implications, but rather the expenditure necessary to address the needs identified.

The same participant asked how a consumer would know which products contain nanomaterials or are produced using nanotechnology; she wondered whether NNI or EPA is involved in such communication. She expressed particular interest in products containing nanomaterials that could enter the sewage system. Dr. Merzbacher replied that, depending on one's definition of nanomaterials, many things could be said to contain nanoparticles. Simply indicating that a product contains or may release nanoparticles is not helpful. It is important to develop a basis for identifying the hazard of specific nanomaterials. At this early stage, little information on the real risk is available.

Department of Energy User Facilities for Nanoscale Science: National Resources for Researchers
Altaf Carim, Office of Basic Energy Sciences (BES), DOE

The DOE, one of the original participants in NNI, provides major funding for nanoscale science, engineering, and technology. The FY 2008 budget request includes more than $285 million for nanotechnology through DOE's Office of Science, which supports both fundamental research and major user facilities. The energy and environmental grand challenge areas were identified from the start of the NNI in FY 2001, and these are mission areas for DOE. In addition, a major NNI- and DOE-sponsored workshop in 2004 identified key research targets and foundational themes for energy-related nanoscience.

The mission of the BES is to: (1) foster and support fundamental research to provide the basis for new, improved, environmentally conscientious energy technologies; and (2) plan, construct, and operate major scientific user facilities for "materials sciences and related disciplines" to serve researchers from academia, federal laboratories, and industry. BES Scientific User Facilities include five Nanoscale Science Research Centers (NSRCs): the Center for Nanoscale Materials at Argonne National Laboratory, the Molecular Foundry at Lawrence Berkeley National Laboratory, the Center for Functional Nanomaterials at Brookhaven National Laboratory, the Center for Nanophase Materials Sciences at Oak Ridge National Laboratory, and the Center for Integrated Nanotechnologies at Sandia National Laboratories and Los Alamos National Laboratory. The NSRCs are research facilities for the synthesis, processing, analysis, and characterization of nanoscale materials. They provide specialized equipment, unique tools, and dedicated support and scientific staff. The NSRCs are operated as user facilities and are available to all researchers, with access determined through peer review of proposals. There is no user fee for precompetitive, nonproprietary work leading to publication; however, costs are recovered for proprietary work. All NSRCs are co-located at DOE National Laboratories with existing major user facilities, including synchrotron radiation light sources, neutron scattering facilities, and other specialized facilities.

In addition to the NSRCs, other user facilities are available, including X-ray scattering, neutron scattering, and electron scattering facilities. The BES light sources—including the Advanced Light Source, Advanced Photon Source, National Synchrotron Light Source, and Stanford Synchrotron Radiation Laboratory—are major user facilities that have a keen interest in nanoscale science. The BES neutron scattering centers include the Intense Pulsed Neutron Source; the Manuel Lujan, Jr. Neutron Scattering Center; the High-Flux Isotope Reactor; and the Spallation Neutron Source. The BES electron scattering user facilities include the National Center for Electron Microscopy at Lawrence Berkeley National Laboratory, the Electron Microscopy Center at Argonne National Laboratory, and the Shared Research Equipment Program at Oak Ridge National Laboratory.

More information on DOE nanoscience is available at http://nano.energy.gov; information on the DOE–BES scientific user facilities is available at http://www.sc.doe.gov/bes/BESfacilities.htm.

Discussion

Dr. Terry Gordon remarked that he had experienced difficulty finding information on the NNI Web Site. He e-mailed three contacts listed on one agency's Web site and never received a response. He asked if a central person is available who could provide advice or information to researchers. Dr. Carim suggested that Dr. Gordon contact a nanoscale user facility directly to ask if he may submit a proposal or to determine if a proposed activity is appropriate for that facility.

Dr. Jacob McDonald recalled an experience similar to Dr. Gordon's. Researchers need an improved interface with the user facilities. In particular, they need someone who can show them how to integrate the tools available at the user facilities into their research. He added that it is very difficult to develop a proposal if one does not yet know how to integrate the tools. Dr. Carim agreed that this is a problem. Facility staff members are eager to work with researchers on how to apply the tools to their research; however, they may not understand all of the fields that their capabilities might serve. Similarly, researchers working in those fields may not be sure which instruments might be useful for their research. He agreed that this is an interface problem and welcomed any suggestions for resolving it.

The National Science Foundation—Discovery, Innovation, and Education in Nanoscale Science and Engineering

Cynthia Ekstein, Chemical, Bioengineering, Environmental, and Transport Systems Division, NSF

Funding for nanoscale science and engineering (NSE) research has increased since FY 2000. NSF's FY 2008 request of $390 million for NSE research funding is one-quarter of the total federal request and one-twelfth of world investment in this type of research. Of NSF's FY 2008 budget request for NSE research, 16.1% is intended to address societal dimensions of nanotechnology, and 7.4% is specifically for nanotechnology EHS research. NSF supports fundamental research in seven program component areas; infrastructure establishment through about 3,500 active projects, including 24 large centers, user facilities, and multidisciplinary teams; and training and education affecting more than 10,000 students and teachers per year. Nanotechnology research and development involves ethical, legal, and social issues. NSF funding priorities for 2007–2008 for knowledge creation, infrastructure, and education include: (1) new measurement methods and instrumentation to characterize nanoparticles and other nanostructured materials and nanosystems, as well as their potential implications; (2) physical-chemical-biological processes of nanostructures dispersed in the environment, including transport phenomena of nanoscale aerosols and colloids from sources to exposure settings and the interaction of nanomaterials with cells and living tissues; (3) safety in nanoscale manufacturing of materials and systems; (4) separation of nanoparticles from fluids; (5) development of experimental and simulation user facilities; and (6) educational programs for nano-EHS. NSF, EPA, NIOSH, NIEHS, and other agencies have jointly supported nanotechnology research for 3 years; NSF also supports research through its Small Grants for

Exploratory Research. Information on NSF-supported nanotechnology research is available at NSF's NNI Web Site, http://www.nsf.gov/nano.

Discussion

A participant asked whether NSF-supported nanotechnology research is available on the NSF Web Site. Dr. Ekstein responded that it is available on the NSF Web Site under "Awards."

The National Institute for Occupational Safety and Health Nanotechnology Program
Vladimir Murashov, Office of the Director, NIOSH

NIOSH is the federal agency responsible for conducting research and making recommendations for the prevention of work-related injury and illness. In 2004, NIOSH created the Nanotechnology Research Center in response to public concern over nanotechnology implications. The NIOSH Nanotechnology Program has developed four strategic goals. The first goal is to understand and prevent work-related injuries and illnesses potentially caused by nanoparticles and nanomaterials. NIOSH is addressing this goal via research on risk assessment and risk management of nanotechnology in the workplace, including toxicology, metrology, control technology, exposure assessment, medical surveillance and guidance, and safety research. The report, *Progress Toward Safe Nanotechnology in the Workplace*, released in 2007, addresses research progress in 10 key areas, research gaps, continuing project plans, and opportunities for collaboration. The second strategic goal of the NIOSH Nanotechnology Program is to promote healthy workplaces through interventions, recommendations, and capacity building. NIOSH is addressing this goal in a number of ways. For example, the NIOSH field team partners with employers to assess exposures in the workplace and the effectiveness of control technologies in the mitigation of those exposures. In addition, NIOSH has developed best practice guidelines for the workplace in the regularly updated report, *Approaches to Safe Nanotechnology: An Information Exchange with NIOSH*. The third strategic goal is to enhance global workplace safety and health through national and international collaboration on nanotechnology. To achieve this goal, NIOSH is engaging in a number of activities, including: (1) collaborations with companies; (2) participation in interagency working groups; (3) participation in the International Organization for Standardization TC 229 Nanotechnology Working Group on Health, Safety, and Environment; (4) collaboration with the Organisation for Economic Co-operation and Development (OECD); and (5) collaboration with the World Health Organization. The fourth strategic goal of the NIOSH Nanotechnology Program is to conduct research to prevent work-related injuries by applying nanotechnology products. To achieve this goal, NIOSH is examining the application of nanotechnology and nanomaterials to the development of filters, sensors, and protective clothing for occupational safety.

NIOSH Nanotechnology Program funding has increased to more than $6 million in 2007; this includes funding for extramural programs, which has remained steady at approximately $1 million per year. NIOSH engages in intramural activities related to nanotechnology, including: (1) the National Occupational Research Agenda: Nanotechnology Safety and Health Research Program; (2) the NIOSH Nanotechnology Research Center; (3) the Nanotechnology Research Supplement; and (4) Nano-related Division Projects. NIOSH also funds nanotechnology research through research grants, joint RFAs, and contracts to address specific needs. Information on NIOSH extramural programs can be found at http://www.cdc.gov/niosh/oep/ and at http://www.grants.gov. Since 2004, NIOSH has been engaged with EPA, NSF, and NIEHS in the joint RFA, "Nanotechnology Research Grants: Investigating Environmental and Human Health Issues." From this RFA, up to $8 million has been spent each year to support 15–25 research grants and exploratory grants, with up to $1 million per year from NIOSH. Research funded by NIOSH addresses the Institute's mission to provide leadership in preventing work-related illnesses and injuries. In FY 2007, NIOSH has worked jointly with the National Institutes of Health (NIH) and EPA on an NIH-led RFA, "Manufactured Nanomaterials: Physico-chemical Principles of Biocompatibility and

Toxicity." From this RFA up to $4.1 million per year will support 10–15 research grants and exploratory grants, including up to $0.5 million from NIOSH.

National Institute of Environmental Health Sciences Activities on Nanotechnology: Nanoscale Science and Toxicology
Nigel Walker, NIEHS, NIH

Nanotechnology activities at NIEHS include research conducted or funded by NIEHS. Researchers in the Division of Intramural Research (DIR), such as those in the National Toxicology Program (NTP), investigate the applications of nanotechnology and characterize nanomaterials. Materials characterized by the NTP are available to researchers for collaborative efforts. DIR investigator-initiated research addresses the application of nanotechnology in EHS. The NTP's areas of emphasis include: (1) exposure and dose metrics; (2) internal dose–pharmacokinetics in biological systems; (3) early biological effects and altered structure or function; and (4) adverse effects related to exposure to nanomaterials. Some common issues and recommendations regarding experimental strategies emerged from several workshops and reports, including the NTP Workshop on Experimental Strategies in 2004 and the International Life Sciences Institute (ILSI), Risk Science Institute (RSI) report (Oberdorster, et al. *Particle and Fibre Toxicology* 2005;2:8). In particular, it is important to traverse the continuum of human relevance and determine how *in vitro* and *in vivo* work should be integrated and to consider whether the materials being studied are the same materials to which humans ultimately will be exposed. The scientific focus of the NTP Nanotechnology Safety Initiative is to identify key physical–chemical features that govern nanomaterial safety. Materials currently under evaluation by NTP include quantum dots (QDs), titanium dioxide (TiO_2), carbon fullerenes, nanoscale silver, multi-walled carbon nanotubes (MWNTs), nanoscale gold, and dendrimers. The NTP uses an open process whereby any member of the public can nominate nanomaterials and other environmental agents to be evaluated by NTP for toxicity. More information on NTP's nanotechnology work can be found at http://ntp.niehs.nih.gov/go/nanotech.

Research is funded by NIEHS through the Division of Extramural Research and Training. Extramural research regarding enabling technologies addresses the applications of nanotechnology, including the development of: (1) deployable environmental sensors for a broad range of environmental exposures; (2) biological sensors to link exposure with disease etiology; (3) intervention devices, such as drug delivery devices and other therapeutic nanoscale materials; and (4) remediation devices, including primary disease prevention through the elimination of exposure. Extramural research funded in the area of the fundamentals of biological response has included research funded under the FY 2006 joint solicitation among EPA, NSF, NIOSH, and NIEHS, "Human Health Effects of Manufactured Nanomaterials." NIEHS funded three applications at $400,000 per year for 3 years on transmembrane transport, cardiovascular toxicity, and oxidative stress. In addition, NIEHS is the lead agency on the joint solicitation with NCI, National Eye Institute, the National Human Genome Research Institute, the National Institute of Dental and Craniofacial Research, the National Institute of General Medical Sciences, EPA, and NIOSH in FY 2007, "Manufactured Nanomaterials: Physico-chemical Principles of Biocompatibility and Toxicity." Approximately 10 grants will be funded from this RFA.

Through the NanoHealth Initiative, NIEHS is taking the next step by building on its investment and core competencies and partnering for integrated research success. The scope of the NanoHealth Initiative is to examine the fundamental physicochemical interactions of engineered nanomaterials (ENMs) with biological systems at the molecular, cellular, and organ level, as well as associated pathophysiologic processes. The rationale behind this initiative includes the acquisition of new knowledge of molecular, cellular, and organ system biology and the identification of clinically relevant properties of ENMs. This initiative is critical for the design of ENMs with maximum human and environmental biocompatibility and safety and will establish the scientific foundation of an emerging science.

Discussion

Ms. Patricia Weggel expressed concern about the communication of risk to researchers who work with nanomaterials, such as those who collaborate with and obtain materials from DIR, through material safety data sheets or other means. Manufacturers are not required to indicate whether nanomaterials are present on material safety data sheets and university and EPA researchers sometimes do not realize how to protect themselves. Dr. Walker agreed that communication of risk to researchers is an area of concern, but this is not something that NTP is addressing.

Dr. Bellina Veronesi noted that she agreed with Dr. Walker about the need to physically characterize nanoparticles because this work is necessary to link physical characteristics to a biological event. Unfortunately, most characterizations are conducted in an environment very different from that of actual biological exposures. Characterization of particles in environmental media can differ substantially from that conducted in distilled water; therefore, nanomaterials should be described in more realistic environments. Dr. Walker replied that NTP has encountered difficulty determining how to quantitate modes of action in biological tissues. Further, agencies such as NIOSH, the Occupational Safety and Health Administration, and EPA will have to address bulk materials. It is important to integrate information about these materials in both kinds of environments to see how the material changes between the raw state and a biological state. Characterization conducted only in a biological environment may not be relevant for NIOSH, for which the occupational hazard is important. The ILSI–RSI report included a useful table of the types of analyses needed at different levels, including dry state or bulk state and *in vitro* or *in vivo* studies. It is important to provide characterizations at all of these levels until the key determinants of biocompatibility are known.

Dr. Warren Layne asked whether the DIR has found that every minor modification of each nanoparticle must be characterized to gain sufficient understanding of the material's properties. Dr. Walker replied that each nanoparticle is different; a nanoparticle with three different surface characteristics may demonstrate three different half-lives, even though all three might be considered to be the same nanoparticle. Dr. Layne asked about ASTM International's efforts to develop standards related to nanoparticles. In light of the differences among particles and the effects of minor modifications to particles, he wondered how a particular nanoparticle under investigation could be compared to a single standard nanoparticle. Dr. Walker said that an upcoming NIST-sponsored workshop will address development of standard materials. Such standards would be useful in that one could compare particular characteristics of a nanoparticle to a known standard, such as a size or colloidal reference; however, it would not be possible to develop standards for every known particle. Dr. Layne asked whether the NTP has found that surface area is a primary determinant of a particle's effects. Dr. Walker said that this may be the case for some types of materials, but in general this is not yet known.

Dr. Zubair Saleem asked whether any of the agencies represented at the workshop have considered the disposal of these materials in terms of sustainability or recycling. Dr. Walker said that this sounds like a question about LCA and post-consumer use. Dr. Nora Savage added that this is a major component of ORD's Nanotechnology Research Strategy, which soon will be released for Agency review, and then will be released to the general public for comment.

Office of Research and Development Introduction
George Gray, ORD, U.S. EPA

EPA recognizes that nanotechnology may have benefits for society, such as enhanced products and processes, reduced waste, and reduced energy use. The potential benefits of nanotechnology may make it integral for addressing environmental challenges. To ensure that such benefits are realized, EPA is funding research in the area of nanotechnology applications. Since 2001, EPA has awarded about 35

grants and has spent approximately $12 million to fund research on the environmental applications of nanotechnology. In addition, it is important to consider the potential environmental and public health effects of the widespread use of this technology. The STAR Program first funded research on the implications of nanotechnology in 2004; now the STAR Program has awarded about 50 grants totaling $10 million to study implications. Implications research topics include: (1) exposure; (2) LCA; (3) risk management; and (4) fate, transport, and transformation. EPA joins with other agencies to produce joint RFAs on the environmental effects of manufactured nanomaterials. In addition, EPA's SBIR program, which works primarily with the private sector, has awarded more than 32 contracts worth in excess of $3 million for small businesses to develop and bring to market nanotechnology-related products.

EPA must be proactive in identifying critical research needs. Program offices will be faced with policy questions related to nanomaterials; as the Agency's science and technology arm, ORD must be sure that science informs regulatory decisionmaking. For that reason, ORD released the *Nanotechnology White Paper* (EPA 100/B-07/001) in February 2007. The white paper, developed by an intra-Agency team, identifies key research needs in the area of nanotechnology and outlines a Nanotechnology Research Strategy to prioritize these needs. The Nanotechnology Research Strategy will be released for public comment in fall 2007. EPA will ask the scientific community if the Agency has effectively identified and prioritized the research needs and the best means to achieve them. The Agency also will consider how to mitigate identified problems and how to manage risks.

The extensive cooperation and communication across the federal government in the area of nanotechnology will result in more rapid progress toward addressing nanotechnology implications. EPA also is very active internationally, for example by leading two working groups for the OECD on the health and safety implications of manufactured nanomaterials. In addition, EPA is working with the Department of State, NSF, NIOSH, NIEHS, and DOE to develop an international RFA.

Nanotechnology holds a great deal of promise, but the American people expect federal agencies and the academic community to be able to reap the benefits of this technology while minimizing risks. This workshop will provide an opportunity to share research results, spread knowledge, find opportunities for collaboration, and recognize the real potential of this technology.

Discussion

Dr. Richard Wiggins commented on the potential for public perception to hinder good science and good policy. He asked whether anyone has considered looking at human behavior as these technologies are developed and applied. The manner in which one communicates with the public is just as important as the actual science. Dr. Gray agreed that this is a good point and said that federal agencies and researchers are interested in science, technology, and problem-solving. It also is important, however, to effectively communicate about nanotechnology so people understand what is and is not known. Studies of risk perception regarding nanotechnology are beginning to appear. He asked Dr. Merzbacher whether she is aware of work in the federal government focusing on communication. Dr. Merzbacher agreed that this is an important issue. In addition to the NEHI Working Group, another interagency group under the same high-level subcommittee addresses public engagement and communication.

A participant asked whether the U.S. Geological Survey (USGS)—which works with EPA on environmental monitoring and other programs related to the occurrence of contaminants in the environment—is conducting nanotechnology research or participating in any interagency nanotechnology efforts. Dr. Sarah Gerould responded that the USGS has been involved in a number of interagency working groups on nanotechnology. The USGS has a budding research program in nanotechnology and is seeking further collaboration.

The Science To Achieve Results Research Program in Nanotechnology: Deepening Our Understanding of the Environmental Aspects of Engineered Nanomaterials
Chris Saint, ORD, U.S. EPA

Established in 1995, the STAR Program is the extramural funding arm of EPA's ORD. Its mission is to include universities and nonprofits in EPA's research program to ensure that the highest quality science supports sound decisionmaking. The STAR Program awards about $66–100 million annually and currently is managing about 1,000 active research grants and fellowships. Each year the STAR Program receives 3,000–3,500 grant applications and makes about 300 new STAR awards.

EPA is interested in nanoscale materials for a number of reasons, including the following: (1) the unique chemical properties of nanoscale materials makes traditional risk management techniques and regulations unsuitable in many situations; (2) these materials have potential environmental applications, such as cleaning up past environmental problems, improving present processes, and preventing future environmental problems; (3) the Agency has regulatory responsibilities because these products are in the marketplace and may pose risks to human health, the environment, or both; and (4) opportunities exist to maximize the environmental benefits and minimize impacts from the beginning, as new technologies are developed. Specific areas of interest for the STAR Program in nanotechnology include research on implications (e.g., potential toxicity; potential exposure; fate, transport, and transformation; and bioavailability and bioaccumulation) and applications (e.g., pollution remediation and treatment, pollutant or microbe monitoring and detection, and the development of environmentally benign processes for pollution prevention). The STAR Program began by funding exploratory research, primarily on applications of nanotechnology, in 2001; the program shifted to exploratory research on the implications of nanotechnology in 2003. The STAR Program began to collaborate with other agencies to solicit proposals on environmental and human health effects through two RFAs issued in 2005 (with NSF and NIOSH) and in 2006 (with NSF, NIOSH, and NIEHS). In 2007, the STAR Program has collaborated with NIEHS to solicit research proposals on the physicochemical principles of biocompatibility and toxicity; the STAR Program also has collaborated with NSF and DOE to solicit proposals on environmental fate, transport, transformation, and exposure research. Other programs managed by NCER include the Greater Research Opportunities program, which has released two RFAs related to nanotechnology (Detection and Monitoring in 2007 and Applications in 2004), and SBIR, which has solicited research on Applications for Environmental Monitoring and Pollution Control in 2001, 2003, and 2004.

This workshop is the fourth in a series of workshops, but is the first truly interagency workshop in which EPA has been involved. This workshop is intended to create novel interactions within the research community and to help federal agencies begin to target research at crucial needs for EPA, other agencies, and the public. More information about nanotechnology research funded by NCER can be obtained from http://www.epa.gov/ncer/nano.

Discussion

A participant asked how the STAR Program will facilitate international collaborations. Dr. Saint explained that, unfortunately, EPA cannot legally provide grants to agencies or institutions outside the United States. The Agency can, however, provide funding to an institution that is cooperating with another institution or agency outside the United States; this is the kind of collaboration the STAR Program encourages. Dr. Savage added that EPA attempted to work with the European Commission on a joint RFA, but this was unsuccessful for logistical reasons. The Agency now is attempting to work on a similar collaborative effort for the future. EPA understands that international cooperation is critical, but EPA, other U.S. agencies, and the NNI must find the best means by which to achieve this kind of cooperation.

RESEARCH PROJECT PRESENTATIONS

Removal and Toxicity of Nanomaterials in Drinking Water
Paul Westerhoff, Arizona State University

The overall goal of this study is to understand the fate and significance of nanomaterials in drinking water. The objectives of this research project are to: (1) characterize the fundamental properties of nanomaterials in aquatic environments; (2) examine the interactions between nanomaterials and organic pollutants and pathogens; (3) evaluate the removal efficiency of nanomaterials by drinking water unit processes; and (4) test the toxicity of nanomaterials in drinking water using a cell culture model system of the epithelium. The researchers used a multidisciplinary approach, including experiments to identify the fundamental uniqueness of nine nanomaterial properties and toxicity, as well as applied experiments to elucidate the fate and reactions involving nanomaterials in drinking water treatment plants. The researchers found that most commercial metal oxide nanoparticles occurred primarily as aggregates in water, but QDs did not aggregate in water. Ionic strength and ionic composition affected further nanoparticle aggregation in water, but this depended on nanoparticle surface chemistry. Natural organic matter (NOM) stabilized nanoparticles in water. During simulated water treatment, alum coagulation and membrane filtration removed most, but not all, nanoparticle mass. The researchers also found that nanoparticles can be toxic to Caco-2 epithelial cells, can affect epithelial layer morphology, and may affect epithelial layer function. In addition, some nanoparticles penetrated confluent epithelial cell monolayers. This study considers the physical, chemical, and biological implications of nanomaterial fate and toxicity in systems and will provide insight into the potential for nanomaterials to be present and to pose health concerns in finished drinking water.

Discussion

Dr. Navid Saleh asked whether the researchers used the same medium both in particle size characterizations and in exposure of cells to nanoparticles. He suggested that one must use a standard method of characterization to determine how the physical behavior of nanoparticles in aquatic environments affects toxicology. Dr. Westerhoff replied that he and his colleagues used dynamic light scattering (DLS) at different concentrations in distilled water and in the medium used for exposure of cells to most of the nanoparticles studied. For QDs, researchers modified the medium by using only phosphate buffered saline. The DLS work discussed in the first part of the presentation used distilled water; this does not represent the particle size during the toxicity test. The researchers do have that information; in fact, even more significant aggregation occurs in the growth medium used in the toxicity test.

Dr. Saleh then commented on the finding that increasing the TiO_2 to 1,000 ppm results in fewer particles penetrating the membrane. He asked whether particle size or concentration might have played a role in this result. Dr. Westerhoff clarified that he presented a percentage of particles passing through the membrane, not absolute numbers of particles. He then explained that he had primarily intended to contrast titanium with cadmium (Cd). At a high concentration of particles (1,000 ppm), 2.2% of TiO_2 nanoparticles pass through the cell monolayer, whereas, at a relatively low particle concentration (1 ppm), 34% of Cd QDs penetrated the cell monolayer.

Pulmonary and Systemic Biocompatibility of Inhaled Carbon Nanotubes
Jake McDonald, Lovelace Respiratory Research Institute

Previous research, published in 2004, showed that instillation of carbon nanotubes resulted in significant lung tissue damage, up to and including death, in very short time scales. In the present research project, investigators hypothesized that, in contrast to instillation, inhalation of carbon nanotubes would not cause

pulmonary injury or inflammation after high-dose exposures. Researchers characterized MWNTs and developed a whole-body inhalation system capable of delivering MWNTs to the air breathed by rodents. Researchers exposed mice to control air or to respirable MWNTs for 7 or 14 days, examined lungs for indications of inflammation, and assessed the systemic response. Mice exposed to MWNTs via inhalation showed unremarkable pulmonary inflammation and pathology even at high doses. Inhalation of MWNTs, however, caused systemic immunosuppression, characterized by reduced T-cell-dependent antibody response to an antigen and suppressed T-cell proliferative ability in the presence of the mitogen, Concanavalin A. Researchers found no change in gene expression in the lungs of MWNT-exposed mice; however, they found large increases in IL-10 and in NQ01 mRNA levels in the spleens of exposed animals, as well as an increase in prostaglandin-associated enzymes. In several other environmental or occupational exposures, the researchers have found similar systemic immune function changes that are not accompanied by pulmonary effects. The immune responses found in this study are likely not unique to MWNTs.

Discussion

Dr. Igor Linkov asked whether the researchers have attempted to run a computational fluid dynamics model with respect to particle size distribution. Dr. McDonald responded that they have not run such a model but would be willing to collaborate with other investigators who are experienced in that approach.

Dr. Gayla Orr raised the issue of potential artifacts due to experimental conditions. She suggested that the specific physical and chemical properties of nanomaterials (e.g., the large surface area-to-mass ratio) make them more reactive; once agglomerated, these materials no longer have the same properties. She asked, therefore, how nanoparticles might produce the same effects regardless of agglomeration. Dr. McDonald clarified that he has not concluded that smaller particles are the most harmful; therefore, these findings are not necessarily artifacts of the experimental conditions. Another participant added that each aggregate has many nanofaces, so aggregation does not necessarily end the effects or reactivity of nanomaterials.

Pharmacokinetics and Biodistribution of Quantum Dot Nanoparticles in Isolated Perfused Skin
Nancy Monteiro-Riviere, North Carolina State University

The objective of this research project is to assess potential health effects—specifically, dermal absorption and cutaneous toxicity—of manufactured nanomaterials in skin. The researchers asked the following questions: (1) Do nanoparticles penetrate the skin? (2) Do such particles preferentially locate in the lipids of the stratum corneum? (3) Can nanoparticles gain access to tissue spaces, a prerequisite for systemic toxicity? Researchers used QDs with various surface coatings, including polyethylene glycol (PEG) and carboxylic acids (COOH). They used flow-through diffusion cells and laser scanning confocal microscopy to assess QD penetration through porcine skin. Flow-through diffusion cells showed penetration of QD621 only in the upper stratum corneum layers of skin. This is in contrast to studies with QD565 and QD655 that showed slight coating-dependent epidermal penetration. In the QD621 infusion study, COOH-coated QDs showed greater tissue extraction than PEG-coated QDs. Images indicate aggregation of infused QDs in the skin vasculature, and transmission electron microscopy (TEM) localized QD621 within the capillary walls. A pharmacokinetic model of arterial-venous extraction and tissue biodistribution of QDs was developed based on a model previously used to quantitate platinum distribution in the same experimental system. Significant arterial-venous QD extraction was observed at all doses, with COOH QDs showing greater predicted tissue deposition; this confirms results of the confocal studies. Researchers found an approximately 90-minute periodicity in arterial extraction, an observation not seen after chemical infusions. Such periodicity could lead to tissue redistribution on chronic exposure, as has been found by other investigators. These data begin to define nanomaterial characteristics that correlate to tissue uptake and persistence. The results are important for risk assessment

and drug delivery because they suggest that QDs not specifically targeted for medical applications can biodistribute to tissues, have unique pharmacokinetic patterns of arterial extraction, and may cause adverse effects.

Discussion

Dr. Layne asked whether these findings have relevance to inhalation exposure in humans. Dr. Jim Riviere recalled the potential systemic effect after pulmonary exposure found by Dr. McDonald and noted that this would occur through redistribution. Although it is not yet known how the nanomaterials redistributed, the potential for such redistribution is important for an understanding of the dose-to-effect pathway. Dr. Monteiro-Riviere added that QDs are used in infusion for medical imaging, and this study shows that the QDs can disperse through the capillaries.

Metal Nanoparticle Tissue Distribution Following* In Vivo *Exposures
Alison Elder, University of Rochester

Studies of ultrafine particles have demonstrated extrapulmonary translocation, but it is not yet known which particle properties affect the tissue distribution of nanosized particles. In the last year of this project, the focus is on the biodistribution and fate of engineered nanoparticles administered via the respiratory tract or systemically. The researchers hypothesized that the tissue distribution of nanomaterials following respiratory tract or systemic exposure is a function of the particles' surface properties. Researchers exposed rats to QDs (with PEG-, PEG-amine-, or COOH-conjugated surfaces) or colloidal gold particles (coated with rat serum albumin or PEG) via intratrachial microspray (ITM) or intravenous injection. Researchers characterized the inflammatory response because inflammation (as determined by percentage of lavage fluid neutrophils) can significantly alter the translocation of nanoparticles between the lung and the blood. They found that inflammation in QD-exposed rats did not differ from that of controls. In contrast, the PEGylated colloidal gold particles caused significant increases in neutrophils when delivered via ITM. The researchers then determined Cd content and gold content in lung and extrapulmonary tissues. They concluded that nanoparticles delivered via the lower respiratory tract are translocated to extrapulmonary tissues, but this is highly dependent on particle physicochemical characteristics. They also found that small amounts of nanoparticles can be retained in brain tissue following a single exposure, but this is dependent on particle physicochemical characteristics and the portal of entry. In the future, the researchers plan to more thoroughly evaluate the kinetics of nanoparticle translocation and to determine in which cells and subcellular structures nanoparticles are localized. In addition, researchers hope to characterize the translocation of particles to the central nervous system (CNS) as a function of the particle surface and its interactions with endogenous proteins; they also plan to characterize the elimination of nanoparticles from the CNS.

Discussion

A participant suggested that when they characterize the translocation of particles to the CNS, the researchers should examine the basal ganglia because they are known to concentrate metals.

Another participant observed that the researchers found very different responses from QDs and gold particles of similar size and coating (PEG). He asked whether the characteristics or amounts of surface coatings might help explain these differences or whether there are other explanations for the differences between PEG-coated QDs and gold particles. Dr. Elder responded that she had initially thought that these differences could be explained by differences between QDs and gold in core chemistry. This may be too simplistic however; the answer may have to do with protein interactions. The same participant asked if all tissues were perfused. Dr. Elder explained that the tissues in this study were not perfused because perfusion can increase the variability of results across animals or across tissues within one animal; this

could interfere with the interpretation of results. The best way to assess the contribution from blood in different tissues is to look for particles in the endothelium and in the tissues themselves to determine where the particles are localizing.

Dr. Veronesi asked why the researchers think that the nanoparticles would leave the brain. Dr. Elder answered that she does not know if they leave the brain. She added that she hopes to address that question more specifically in the future by, for example, sampling cerebrospinal fluid in different regions of the brain. This is a critical issue because of Dr. Veronesi's work showing that, in the presence of ambient particulate matter, neurotoxicological effects may be attributable to the particulate fraction. It is necessary to determine where the particles are going and how long they are staying there.

Bioavailability, Toxicity, and Trophic Transfer of Manufactured ZnO Nanoparticles: A View From the Bottom

Jason Unrine, University of Georgia

The objectives of this research project are to: (1) evaluate the bioavailability and toxicity of manufactured zinc oxide (ZnO) nanoparticles to model soil bacteria (*Burkholderia vietnamiensis* and *Cupriavidus necator*) and the model detritivore *Caenorhabditis elegans* as a function of particle size, compared with aqueous Zn^{2+}; (2) evaluate the ability of manufactured ZnO nanoparticles to be transferred from one trophic level to the next as assessed in the simple food chain consisting of pre-exposed *B. vietnamiensis* and *C. elegans*; and (3) evaluate the additive, synergistic, or antagonistic effects of manufactured ZnO nanoparticles on the toxicity of Cu^{2+} to *B. vietnamiensis* and *C. elegans*. The researchers hypothesized that: (1) the bioavailability and toxicity of manufactured ZnO nanoparticles would increase with decreasing particle size; (2) the toxicity of ZnO nanoparticles to model soil bacteria and *C. elegans* would be lower than an equivalent concentration of dissolved Zn^{2+}; (3) the bioavailability and toxicity of ZnO nanoparticles introduced via trophic transfer would differ from direct exposure; and (4) ZnO nanoparticles would alter the bioavailability and toxicity of dissolved metals. The researchers found that size determination is a critical issue and that TEM may not be the best method for ZnO nanoparticle size determination. Further, acetate controls ZnO nanoparticle reactivity and passivates surface sites; the removal of acetate leads to aggregation of ZnO nanoparticles but promotes surface reactivity. Regarding nanoparticle–bacteria interactions, researchers found no significant difference in growth rates of bacteria in the presence of aqueous Zn^{2+} versus ZnO nanoparticles. Acetate use rates, however, were higher in the presence of aqueous Zn^{2+} compared with ZnO nanoparticles. Researchers found some evidence for zinc bioavailability from Zn^{2+} but not from ZnO nanoparticles. In addition, cells with compromised membranes were more strongly associated with ZnO nanoparticle treatment than with Zn^{2+} treatment. Therefore, even though growth rates in the presence of ZnO nanoparticles and Zn^{2+} do not differ, there may be differences in the mechanisms of toxicity. Regarding nanoparticle–nematode interactions, researchers found that the LC_{50} and EC_{50} of ZnO nanoparticles do not differ significantly from those of aqueous Zn^{2+}; however, the mechanisms of toxicity differ. In addition, at zinc concentrations of greater than 100 mg/L, copper toxicity to nematodes is decreased more by ZnO nanoparticles than by aqueous Zn^{2+}. Finally, no significant green fluorescent protein (GFP) was induced by exposure of *C. elegans* to either 100 µM Cd or 500 µM ZnO nanoparticles. In the future, the researchers plan to characterize 80 nm ZnO nanoparticles under various chemical conditions; study the bioavailability, toxicity, and behavior of 80 nm zinc nanoparticles; and continue exposure experiments using Cu^{2+}. In addition, the researchers plan to conduct the following bioavailability and toxicity studies: (1) an investigation of the differences between toxicity mechanisms of $ZnCl_2$ and ZnO nanoparticles in *B. vietnamiensis, Cupriavidus necator,* and *C. elegans*; (2) an examination of the bioavailability and toxicity of ZnO nanoparticles introduced via trophic transfer as opposed to direct exposure; (3) identification of chemical speciation of zinc in concentrated regions in tissues; and (4) an examination of potential transformation of ingested ZnO nanoparticles.

Discussion

Dr. Greg Mayer asked, regarding the metallothionein and GFP work, whether the researchers have any evidence regarding immune function and its upregulation in *C. elegans*. Dr. Unrine responded that he does not have such evidence, but the researchers are aware of experiments with ionizing radiation exposure that might be related to this issue.

Biochemical, Molecular, and Cellular Responses of Zebrafish Exposed to Metallic Nanoparticles
David Barber, University of Florida

The goals of this research project are to: (1) expand the database of acute toxicity of metallic nanomaterials in aquatic organisms; (2) evaluate the role of particle composition and dissolution in gill toxicity; and (3) determine the role of particle surface charge in uptake and retention of nanomaterials in aquatic organisms. To address the first goal, researchers assessed toxicity of nanoparticles and their soluble counterparts to aquatic organisms. To address the second goal, researchers exposed zebrafish to TiO_2, silver, or copper particles and evaluated gill metal uptake, histology, and transcriptional changes at 24 and 48 hours. To address the third goal, researchers examined the uptake and retention of PEG, NH_2, and COOH QDs in *Daphnia*. The researchers found that nanometals can be acutely toxic to aquatic organisms, but they are typically less toxic than their soluble counterparts. Nanoparticles aggregate rapidly once they are introduced into water. Large numbers of nanosized particles, however, are likely to remain in the water column for long periods of time; this may allow for prolonged exposure after a release of nanomaterial into the environment. Changes in particles over time make dosimetry problematic. Results suggest that the effects of some nanometals are not completely explained by dissolution; in particular, the effects appear to depend on particle composition, and this is not a generic (uniform) particle response. Finally, the researchers concluded that particle surface charge influences the uptake of nanomaterials, at least by *Daphnia*. Future work will focus on mechanisms, such as whether particles are entering the gill cell.

Discussion

Dr. Mayer commented that, in the cluster analysis conducted as part of the transcriptional work, silver and copper clustered individually; however, the silver and copper appeared to have extensive commonality except that the copper appeared to have an additional group of upregulated genes. He asked whether those genes are associated with a hypoxia response. Dr. Barber responded that he does not yet know, but he and his colleagues are attempting to address this kind of question. Unfortunately, even though the zebrafish has been studied for a long time, the annotation of the genome is not very complete. The researchers are conducting data-mining to come up with testable hypotheses. Some evidence does exist for a hypoxia-like response.

Acute and Developmental Toxicity of Metal Oxide Nanoparticles in Fish and Frogs
George Cobb, Texas Tech University

The objectives of this research project are to determine the environmental hazard of metal oxide nanoparticles (Fe_2O_3, ZnO, CuO, and TiO_2) in terms of acute and chronic toxicity of these particles to fathead minnows (*Pimephase promelas*) and African clawed frogs (*Xenopus laevis*). The researchers hypothesized that nanoparticle exposure would affect the survival, growth, development, egg hatchability, and metamorphosis of *P. promelas* and *X. laevis*. Researchers have synthesized nanoparticles and have obtained commercial nanoparticles. Acute (96-hour) exposure of *X. laevis* to metal oxide nanoparticles demonstrated developmental effects for one of the nanometal oxides, ZnO (EC_{50} = 8 mg/L). Inhibited growth was observed for *Xenopus* embryos exposed to CuO and ZnO suspensions of greater than 10 mg/L and 100 mg/L, respectively. Scanning electron microscopy showed metal oxide nanoparticles

trapped in ciliated skin cells. This close proximity to the embryo skin may play a role in any observed acute or chronic results. Acute tests for *P. promelas* are just beginning. Chronic tests will include 28-day early life stage tests for *P. promelas* and 10-week exposures for *X. laevis*. Nanoparticles will be kept in suspension in water using aeration- or peristaltic pump-induced water currents. Metal concentrations in water and tissues are being measured via atomic absorption spectrophotometry.

Mechanistic Dosimetry Models of Nanomaterial Deposition in the Respiratory Tract
Bahman Asgharian, The Hamner Institutes for Health Sciences

Accurate health risk assessments of inhalation exposure to nanomaterials will require mechanistic dosimetry models that account for interspecies differences in dose delivered to the respiratory tract. The objectives of this research project are to: (1) measure deposition of nanosized particles in casts of human and rat nasal upper respiratory tract (URT) airways; (2) develop semi-empirical relationships to predict nanomaterial deposition in URT airways; (3) develop respiratory tract deposition models of nanoparticles and nanotubes in humans and rats; (4) measure regional and lobar deposition of nanomaterial in the heads and lungs of rats; and (5) develop a user-friendly software package to implement models and provide rapid simulation capability. The researchers have measured deposition fractions in the nasal airways of humans and rats for particle sizes from 5 to 100 nm. They also have obtained a semi-empirical deposition efficiency formula for humans and rats. The researchers have extended a model of particle deposition in the lung to the ultrafine (nano) size range by including axial diffusion and convective mixing (dispersion). Finally, they have measured lobar and regional deposition of nanoparticles in Long–Evans rats. Development of the software package is in progress.

Discussion

Dr. McDonald observed that in some studies, a 15- or 20-minute exposure will result in as many particles in the gastrointestinal tract as in the lung. He asked whether this might be related to clearance. Dr. Asgharian agreed that this is what his observations suggest. Dr. McDonald asked whether the researchers took any other tissues after exposure that could be measured. Dr. Asgharian replied that they collected lobes, trachea, and head.

Preparation and Application of Stabilized Fe-Pd Nanoparticles for* In Situ *Dechlorination in Soils and Groundwater: Factors Affecting Particle Transport and Reactivity
Don Zhao, Auburn University

The overall goal of this research project is to develop a cost-effective, *in-situ* remediation technology employing a new class of soil-dispersible, iron (Fe)-based nanoparticles for rapid destruction of chlorinated hydrocarbons in soil and groundwater. In Year 2, the researchers completed the following: (1) prepared nanoparticles of various sizes using carboxymethyl cellulose (CMC) as a stabilizer; (2) tested effects of particle stabilization on reactivity; (3) tested transport behaviors of zerovalent iron (ZVI) nanoparticles in porous media; (4) tested degradation of trichloroethylene (TCE) in soils; and (5) pilot-tested *in situ* dechlorination in soils using stabilized ZVI nanoparticles. The researchers developed a method for synthesizing ZVI nanoparticles of controllable size, soil mobility, and reactivity. They found that factors such as CMC molecular weight, the CMC:Fe ratio, pH, and temperature can greatly affect transport and reactivity of nanoparticles. The researchers also found that stabilized ZVI nanoparticles can be delivered and distributed in soils. The nanoparticles can effectively degrade nonaqueous phase liquids in soils and groundwater and may boost biodegradation.

Discussion

A participant noted that one of the unfortunate intermediate products of ZVI and TCE dechlorination is the carcinogen vinyl chloride and asked whether Dr. Zhao had examined this byproduct. Dr. Zhao responded that TCE degradation has two pathways, one biotic and the other abiotic. Degradation by the stabilized ZVI nanoparticles did not lead to the production of vinyl chloride, and TCE is completely degraded to ethene and chloride. However, biological processes can result in vinyl chloride production.

Dr. Layne asked if it would be possible to return to the pilot site or the samples to measure vinyl chloride. Dr. Zhao replied that researchers are still sampling at the pilot site so this would be possible. He predicted that vinyl chloride would not be found there but that, in the second phase (the ZVI-boosted biodegradation phase), vinyl chloride will probably be detectable.

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Engineered Nanoparticles in Environmental Remediation Technology and Implications to Nanoparticle Transport Through the Skin Barrier
Vijay John, Tulane University

The goal of this research project is to develop novel mesoporous materials that act as supports for ZVI nanoparticles used in the breakdown of chlorinated compounds. Dense nonaqueous phase liquids (DNAPLs) are pollutants of concern that are prevalent at contaminated sites. Widely used as a solvent by industry, TCE is a DNAPL that resists biotic and abiotic degradation in natural environments. ZVI can react with TCE through redox chemistry, and nanoscale Fe particles have been found to be most effective in TCE remediation. In a new approach to the environmental remediation of TCE, the researchers are investigating the use of functional Fe–silica submicroscopic particles prepared through an aerosol-assisted route. They have found that: (1) functionalized composite particles significantly adsorb TCE; (2) composite particles are effective in TCE decontamination; (3) composite particles partition to the TCE–water interface; and (4) composite particles have the optimal size characteristics to be effective in transport through sediments. High particle production rates are possible using the aerosol technique. In work related to human health, the researchers have found that nanoparticle penetration pathways through skin are highly dependent on both the initial microstructure and induced conformation changes. They also have found that extensive hydration affects the skin barrier and may allow extremely small nanoparticles to pass through hydration-induced defects in the stratum corneum. This work has implications for human health challenges, such as transcutaneous vaccine delivery.

Discussion

Dr. Riviere commented that there is an extensive literature on lipid biophysics and the effects of hydration. He added that this work is relevant for public health because people go swimming and this hydrates the skin extensively.

Dr. Layne asked how openings in human skin, such as hair follicles and sweat glands, might be relevant to the delivery of nanoparticles through human skin. Dr. John responded that nanoparticles do enter hair follicles but cannot penetrate the follicle to enter the skin. He added that the drug delivery model appears to indicate that sweat glands also are not the primary pathway for entering the skin.

Responses of Lung Cells to Metals in Manufactured Nanoparticles
John Veranth, University of Utah

This research project is based on the hypothesis that, because of their small physical size and large surface area, nanoparticles would increase cellular uptake and the induction of proinflammatory signaling compared with larger particles with the same elemental composition. The researchers predicted that, in comparison with other environmental and occupational agonists, nano-sized metal oxides would have moderate potency in lung epithelial cells. They are using commercially available particles of metal oxides (SiO_2, TiO_2, Fe_2O_3, Al_2O_3, NiO, CeO_2, and ZnO) and are conducting *in vitro* cell culture screening assays and *in vivo* confirmation. The researchers have found that the oxide nanoparticles have low potency in lung epithelial cells compared with soil-driven dusts and vanadium. They concluded that manufactured metal oxide nanoparticles may pose a risk comparable to other ambient and occupational particle types, such as micron-sized crystalline silica. Multiple cell types occur in the lung, however, and vascular endothelial cells are in close proximity to the airspace. Translocation of nanoparticles from the lung is well documented and cardiovascular effects occur in response to environmental air pollution. The researchers currently are investigating responses of endothelial cells *in vitro*. They have found that some types of nanosilica induce proinflammatory signaling in endothelial cells, and now are examining the biochemical mechanisms linking particles to inflammation. Dosimetry is an important consideration with *in vitro* particle studies because responses often are seen only at concentrations much higher than those plausible for inhalation exposure; however, this may be a reflection of cell culture artifacts. Ongoing work includes: (1) continued comparisons between lung epithelial and endothelial cells *in vitro*; (2) the use of specific inhibitors to study cell signaling pathways activated by the more potent types of nanoparticles; and (3) animal exposure via intratracheal aspiration to validate *in vitro* results.

Discussion

Dr. McDonald commented that this study reaffirms that one should not rush to judgment on the relative toxicity of nanoparticles compared with larger particles. With respect to the conclusion that nanoparticles may have potency similar to that of Min-U-Sil silica or silica, he suggested that this may be a bit overstated. Researchers in the Rochester group have compared crystalline (Min-U-Sil) silica to amorphous (nano) silica. They found that the nanosilica was much less potent than Min-U-Sil silica. He added that crystalline silica is quite toxic and is regulated as such. Dr. Veranth agreed that he should have cited that earlier work in addition to the more recent papers.

Dr. Elder commented on the notion of signaling pathways as a possible explanation for the cell responses and noted that those pathways can be activated at the surface of the cell through receptor-mediated processes. She asked whether particles are being taken up by the cells and whether uptake is required for the response of the cells. Dr. Veranth responded that this is not yet known. Some of the receptors are membrane receptors, so they could certainly respond to something outside the cell. He noted that he has done some work with a membrane receptor and the inhibitor suppressed the response. He cited previous research and suggested that particle contact with the cell may be triggering the response, but these particles also are taken up. With soil dust, an inhibitor of phagocytosis did not affect the response, but for nanoparticles we do not have an answer.

Dr. Greg Lowry asked whether Dr. Veranth conducts particle characterizations in the vehicles in which the exposures are conducted. It may be important to examine the state of aggregation, surface charge, functionalization of the surface, and what impacts those factors have on toxicity or other responses in this study. Dr. Veranth replied that he has done some work of this type and has found aggregation. The most relevant model might be to contact the particles first with one surfactant and let them become coated with the lipids and proteins of the surfactant before applying the surfactant to the particles. He has attempted this experiment and needs to do more work in that area. He added that this is one of the limitations of an

in vitro model because of the use of a large amount of a fairly dilute nutrient medium. In contrast, particles in the lung are in contact with a thick viscous surfactant. Dr. Lowry suggested that an understanding of the mechanisms will require an understanding of the properties of the particles; it will be necessary to characterize them in the vehicle and then do the exposure and relate those two conditions.

A Toxicogenomics Approach for Assessing the Safety of Single-Walled Carbon Nanotubes in Human Skin and Lung Cells
Mary Jane Cunningham, Houston Advanced Research Center

The objectives of this research project are to: (1) obtain expression profiles (EPs) of known nanomaterials and unknown nanomaterials, and to compare these EPs to identify toxic effects; and (2) use a systems biology approach to perturb the biological system and reiteratively sample over time or dose. This is a data-driven approach, rather than a hypothesis-driven approach, and involves reverse engineering of cellular pathways. The goal is to create a virtual cell interaction network to predict adverse effects by combining genomics, proteomics, metabonomics, and pharmacogenomics. In previous work with primary human epidermal keratinocytes (dermal exposure route), the researchers found that, with a noncytotoxic dose, the EP of single-walled carbon nanotubes (SWNT) is more similar to the EP of the nontoxic control (CI), and the EP of silicon dioxide (SiO_2) is the most active. With a cytotoxic dose, the EP of SWNT is more similar to the EP of the toxic control (SiO_2), and the EP of CI is most active. In accordance with previous research, the significantly expressed genes in the SiO_2 treatment included genes involved in membrane restoration or remodeling and inflammation or irritation responses. In studies with primary human bronchial epithelial cells (inhalation exposure route), the researchers found that lung cells were not as robust in their long-term growth as skin cells, and it was not possible to perform the complete time course. As with the skin cells, however, there was very low variation between array replicates. The EP of SiO_2 was the most different and the most active, and the EP of SWNT was more similar to the EP of CI. Significantly expressed genes were similar to those found with skin cells and included genes active in inflammation, irritation, and membrane remodeling. Any adverse effects observed with SWNT appear to be limited to local inflammation caused by the physical presence of particulate material. In a principle components analysis comparison of EPs from skin and lung cells, the researchers found the maximum difference between tissue types rather than between types of compounds. The EP of SWNT in both lung and skin cells is similar to that of untreated samples. The researchers observed about 10 times more overall activity in skin cells than in lung cells. In protein expression work, they found that only six proteins were significantly expressed at 24 hours. In miRNA expression work, the researchers found low variability among array replicates. The greatest miRNA expression occurred in the SiO_2 treatment and 71 miRNAs were significantly expressed. Pathway analysis and interpretation of the miRNA expression work is ongoing.

Discussion

Dr. Saleh noted that SWNTs and MWNTs are extremely hydrophobic. He has found that, when bacterial cells are exposed to MWNTs, they are nontoxic if they are not in direct contact with the cell. This is because SWNTs and MWNTs agglomerate and, because the density is so low, they do not settle in any aqueous suspension; instead, they float around and do not make contact with the cells. Therefore, particularly with nanotubes, it is important to be sure that the cells are exposed. He asked how the researchers conducted the exposure. Dr. Cunningham responded that she and colleagues sonicated the nanotubes for an hour, causing a dispersion of individual tubes. A dosage on the order of mg/mL is required to achieve any reduction in viability. In addition, the researchers used serum-free defined media and achieved a heterogeneous suspension of media and nanotubes long enough to treat the cells. After 24 hours, all nanotubes settle on the cells, essentially suffocating them. She had not observed the oxidative stress that others have found, but she noted that she is using a highly purified preparation of nanotubes. Also, they are investigating the use of surfactants to help keep the nanotubes fully dispersed. The first

surfactant in which they dispersed the nanotubes was cytotoxic, and the researchers tried many different surfactants to find one that was nontoxic.

Dr. Subhas Malghan mentioned that Fe is a potential contaminant in the raw material; its removal requires the use of other processes that may change the surface properties of the nanotubes completely. He asked Dr. Cunningham to consider the medical applications of these materials. Dr. Cunningham responded that the carbon nanotubes have been fully characterized, but purification occurs after the manufacturing steps to remove heavy metals. In early work with carbon nanotubes, the large percentage of heavy metals present could explain some of the oxidative stress found in those studies.

Microbial Impacts of Engineered Nanoparticles
Delina Lyon, Rice University

Fullerenes, such as C_{60}, constitute a class of nanomaterials that show potential for medical, industrial, and technological applications. C_{60} is insoluble in water but will form a suspension termed nC_{60} upon extended exposure to water or after introduction to water via a solvent. The researchers examined the effect of nanomaterials on bacteria because the disposal or accidental discharge of nanomaterials could affect microbial ecology and disrupt biogeochemical cycles. In addition, antibacterial activity may be indicative of toxicity to higher level organisms. From the perspective of applications, compounds with antibacterial activity could be used in water treatment or other disinfection. nC_{60} is antibacterial. In eukaryotes, reactive oxygen species (ROS) may mediate nC_{60} toxicity. In prokaryotes, however, the antibacterial activity of nC_{60} persists in the absence of light and oxygen. In this study, researchers explored three possible mechanisms for the antibacterial activity of nC_{60}: (1) physical disruption of the cell membrane; (2) generation of ROS; or (3) production of ROS-independent oxidative stress. The researchers found no conclusive evidence of ROS production or ROS damage in bacteria by nC_{60}. They encourage researchers who previously showed evidence of ROS production or damage to reevaluate their results, as these results may be biased by the ability of nC_{60} to interfere with assays. Findings suggest that nC_{60} acts as an oxidant, possibly requiring direct contact with the cell.

Discussion

Dr. Lee Ferguson noted that the researchers used the tetrahydrofuran (THF) method to produce nC_{60} and asked if they had determined whether residual THF was present in the nC_{60} after the evaporation. Ms. Lyon replied that she and her colleagues had not addressed this issue; however, other groups have not found residual THF. It is possible that the THF is incorporated into the nC_{60} suspension. The researchers attempt to remove all THF, but they also find the same antibacterial activity with stirred nC_{60} (without solvents). Dr. Ferguson continued that he has had problems with evaporating large amounts of THF and using that for toxicity assays because most THF contains stabilizers and oxidation inhibitors that probably would not evaporate. Ms. Lyon clarified that the THF used in this research project is not stabilized.

Dr. Veronesi noted that electron microscopy may help explain the mechanism of antibacterial activity. Ms. Lyon answered that she and her colleagues have attempted electron microscopy but this has proven logistically difficult. They have tried to embed the bacteria in agarose, thinly slice it, and look for nC_{60} particles either inside the cell or elsewhere. They know that nC_{60} likes to sorb to bacteria, but whether it penetrates the membrane is not clear. The nC_{60} particles are as small as 2 nm, so they could be penetrating the membrane because work with QDs has shown that particles smaller than 5 nm can be incorporated into bacteria.

Dr. Ted Henry said that he and colleagues have found that some of the degradation products of THF have been responsible for toxicity in zebrafish. He asked how Ms. Lyon prepared the controls; in particular, he wondered whether the preparation of the controls was the same as that for the THF nC_{60} treatment (i.e.,

evaporation of the THF). Ms. Lyon explained that she and her colleagues did not use THF in the negative controls, but they examined the antibacterial activity initially and did not find toxicity in that condition.

An Integrated Approach Toward Understanding the Inflammatory Response of Mice to Inhaled Manufactured Nanoparticles
Vicki Grassian, University of Iowa

The goal of this research project is to use an integrative approach to determine which physicochemical properties and conditions are important in nanoparticle toxicity. The specific objectives are to: (1) use state-of-the-art techniques to fully characterize a variety of manufactured metal and metal oxide nanomaterials in terms of their size, aggregation state, shape, and bulk and surface properties; (2) determine if engineered nanomaterials are particularly deleterious to health compared with particles from combustion processes that have been more extensively studied; and (3) evaluate the relative health effects of different nanoparticle surface coatings. The researchers found that subchronic inhalation exposure to 5-nm TiO_2 nanoparticles (one of the smallest commercially available oxide nanoparticles) caused an increase in the number of activated macrophages, but mice recovered 3 weeks after exposure. Furthermore, they found no signs of pathological changes in bronchoalveolar lavage fluid or in lung tissue. These particles are pure anatase and their surfaces are truncated by surface hydroxyl groups and adsorbed water under ambient conditions. Thus, no surface coatings are present from manufacturing. They also found that acute inhalation and instillation exposures did not show an effect of surface area for 5- versus 20-nm TiO_2 nanoparticles. Agglomeration state (agglomerate size and porosity) may be the most important factor in these experiments. Studies on metal nanoparticles are underway. Although 25-nm Fe nanoparticles are similar to 5-nm TiO_2 nanoparticles in response, copper nanoparticles show the largest inflammatory response. Metal nanoparticles are coated with an oxide surface layer that will be important in understanding the toxicity of these particles. Bulk and surface characterization shows that these oxide coatings are composed of one or more crystalline phases. Current studies focus on further understanding the chemical characteristics of these oxide layers and how they influence metal nanoparticle toxicity.

Discussion

Dr. Elder agreed with the importance of airborne agglomeration state. She also asked if both the 5- and 20-nm TiO_2 particles had the same crystal structure (i.e., pure anatase). Dr. Grassian clarified that any commercially available particles above 20 nm will contain some rutile, even if they are labeled 100% anatase; this is related to stability and thermodynamics. Below 20 nm one can find pure anatase.

Another participant mentioned the effect of the edges and corners of nanoparticles on their reactivity. He asked whether the researchers had found parallels to silicosis, noting that fresh silica dust is more toxic than stale silica dust. Dr. Grassian answered that she has not seen any findings regarding aging effects. She and colleagues use commercially available materials and always do an independent characterization; however, they have not addressed the aging effect.

Hysteretic Accumulation and Release of Nanomaterials in the Vadose Zone
Tohren Kibbey, University of Oklahoma

Any nanomaterial that is widely used will ultimately enter the environment. The vadose zone may either provide a sink for nanomaterials, preventing their spread throughout the environment, or a long-term contaminant source. The objective of this research project is to study the vadose zone accumulation and release of a wide range of manufactured nanomaterials. The researchers are focusing on an examination of hysteretic interactions with air–water interfaces and specific mineral surfaces. They are assessing adsorption and adhesion affinities with critical liquid–solid and liquid–air interfaces. They also are

evaluating dynamic interactions between nanomaterials and mineral surfaces using saturated deposition and dispersion transport experiments. Finally, they are conducting dynamic hysteretic unsaturated transport experiments to provide detailed information about the effects of wetting and drying history, infiltration, and unsaturated soil behavior on the accumulation and release of nanomaterials. For the work presented, the systems were specifically designed so that saturated transport of nanomaterials would not be a factor. Therefore, any increase in retention seen in unsaturated experiments must be attributable to the formation of the air–water interface. Regarding transport of TiO_2, the researchers have found, using miniature unsaturated transport experiments, that the average mass of nanomaterial retained in the soil cell is much higher for the slowest drainage flow rate investigated than for the faster rates. When these results are normalized for interfacial area, again, the slowest drainage flow rate has the highest retention of nanomaterial. The mass per area of the nanomaterial was found to be approximately constant over a large saturation range, suggesting that adsorption to air–water interfaces is an important mechanism over much of the saturation range. Ongoing and future work using miniature dynamic unsaturated transport experiments includes: (1) the use of multiple drainage–imbibation cycles and an examination of wetting–drying history effects; (2) experiments with changing nanomaterial concentrations; (3) the use of different porous media with smaller grain sizes; and (4) an investigation of more nanomaterials, including those that interact with solid surfaces. The researchers also have begun large-scale experiments, including: (1) unsaturated transport modeling to interpret results of the large-scale experiments; (2) the use of a new column with higher resolution; (3) the use of heterogeneous packings that include a combination of fine and coarse material to determine how the different water contents of the different layers influence the movement of nanomaterials; (4) the use of different drainage and imbibation paths; and (5) the use of more nanomaterials, including those that interact with solid surfaces.

Discussion

Dr. Saleh asked Dr. Kibbey to compare the expected retention of hydrophobic materials, such as carbon nanotubes, to that of the hydrophilic materials used in this study (e.g., TiO_2), in terms of unsaturated transport. Dr. Kibbey explained that he would expect much more retention in the air–water interface for hydrophobic materials. The effect would be greater at lower saturations where there is a greater air–water interface. The challenge would be to disperse a sufficient quantity of nanotubes in water to begin with, and other surface-active chemicals would probably be required to stabilize them.

Dr. Kurt Pennell asked whether the researchers measured surface tension or interfacial tension as another measure of adsorption. Dr. Kibbey replied that they have done this in a few cases, and in some cases the surface tension increases slightly, which is not consistent with adsorption for a dissolved compound. However, it is not clear that the Gibbs adsorption equation can be applied to particle adsorption.

Dr. Saleem asked what kind of water the researchers used. Dr. Kibbey said that they used nanopure water but added ionic strength. Dr. Saleem noted the potential for microbiological activity, but Dr. Kibbey clarified that the time scale of the miniature experiments is on the order of minutes to hours.

The Role of Particle Agglomeration in Nanoparticle Toxicity
Terry Gordon, New York University School of Medicine

The hypothesis of this research project is that the toxicity of fresh (predominantly singlet) carbon nanoparticles differs from that of aged (predominantly agglomerated) carbon nanoparticles. The researchers further predicted that this difference also would apply to metal nanoparticles. The objectives were to: (1) measure the agglomeration rate of carbon nanoparticles; (2) identify whether agglomeration is affected by altering exposure conditions, such as humidity and particle charge; and (3) compare the toxicity of singlet versus agglomerated particles in mice exposed via inhalation. The researchers used a dynamic exposure system to establish the agglomeration of freshly generated carbon nanoparticles at

various distances downstream from particle generation. They then exposed mice to nanoparticles at different stages of particle agglomeration and examined lungs for injury and inflammation. The researchers found a dose–response relationship between exposure to carbon and metal nanoparticles and lung inflammation such that the effects of fresh particles were greater than those of aged particles for carbon particles, but not for copper particles. Humidity and particle charge had no effect on the toxicity of carbon nanoparticles. They found that copper and zinc nanoparticles are more toxic than carbon nanoparticles, and copper nanoparticles are more toxic than zinc nanoparticles. In contrast to carbon nanoparticles, copper particles showed only a small difference between fresh and aged nanoparticles. Differences in response among mouse strains suggest that genetic susceptibility could be involved in the response to nanoparticles.

Discussion

A participant asked whether the carbon source was pure or whether it might have contained metals. Dr. Gordon clarified that he and colleagues used 99.9 or 99.99% pure carbon, zinc, or copper electrodes.

Dr. Patrick O'Shaughnessy remarked that he did not see any results presented regarding the age versus size distribution of particles and asked whether the fresh and aged particles have the same size distribution. Dr. Gordon replied that, in general, the count median diameter of the fresh particles is 10–50 nm and the diameter of the aged particles is 190–250 nm. Dr. O'Shaughnessy asked whether Dr. Gordon was more focused on fresh versus aged as the factor influencing toxicity, rather than differences in size distribution. Dr. Gordon agreed that this was correct and added that he does not predict a substantial change in surface area resulting from agglomeration.

Dr. Henry wondered whether the same kinds of strain differences would occur with other types of toxicants. Dr. Gordon responded that this appears to be the case and offered the examples of cigarette smoke and ozone.

Chemical and Biological Behavior of Carbon Nanotubes in Estuarine Sedimentary Systems
P. Lee Ferguson, University of South Carolina

Carbon SWNTs are hydrophobic and will likely associate strongly with sediments upon entry into the aquatic environment. In sediments, these materials may cause toxicity to benthic, sediment-ingesting organisms and may impact the disposition of persistent and bioaccumulative organic contaminants, such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs). The objectives of this research project are to: (1) determine which factors control the fate of SWNTs in estuarine seawater, sediment, and sediment-ingesting organisms; (2) examine the impact of SWNTs on the disposition of model organic contaminants in estuarine sediments; (3) assess the toxicity of SWNTs to a model deposit-feeding estuarine invertebrate in seawater; and (4) determine whether the presence of SWNTs in estuarine sediments affects the bioavailability of model organic contaminants to suspension- and deposit-feeding estuarine invertebrates. The researchers concluded that SWNTs entering estuaries are likely to associate strongly with suspended particulates and concentrate in sediments during estuarine mixing. Relative to other carbonaceous materials, SWNTs are highly sorptive to hydrophobic organic contaminants (HOCs) and may sequester these compounds in aqueous environments. They found that purified SWNTs are relatively nontoxic to benthic deposit-feeding organisms, but exposure of these organisms to SWNT carbonaceous synthetic byproducts may pose a risk of adverse effects. The consequences of HOC sorption to SWNTs in estuarine sediments for contaminant bioavailability to deposit-feeding organisms are still unclear.

Discussion

Dr. Robert Hurt noted that, because the researchers are studying materials with gel electrophoresis, they are essentially studying a relatively hydrophilic form of nanotubes. He asked whether Dr. Ferguson had considered annealing the products after separation or subjecting them to a process that would reduce them to their more commonly used hydrophobic form. Dr. Ferguson responded that his colleague, Walter Scrivens, has performed re-annealing after purification, but they have not done any experiments with toxicity or sorption after re-annealing. He agreed that these materials are hydrophilic, negatively charged, and have been oxidated. He clarified that the nanotubes themselves do not penetrate the gel; it is the short tubular nanocarbons and the fluorescent nanocarbons that penetrate the gel. The short tubular nanocarbons are aggregates and are much larger than the fluorescent nanocarbons. The researchers have not been able to obtain good TEM images of the fluorescent nanocarbons, and they generally are difficult to analyze. They are not PAHs—or at least they are not small PAHs that can be examined with gas chromatography. He suggested that they might be nanobarrels with very short aspect ratios.

Dr. Mamadou Diallo asked if Dr. Ferguson and colleagues had assessed the effect of organic matter modification. He noted that the effects of divalent cations could explain some of the findings. Dr. Ferguson replied that he added DOM to the nanotubes in the presence of divalent cations and did not see any changes in aggregation; the nanotubes aggregate in the presence of divalent cations whether or not DOM is present. He further clarified that he also conducted the experiments without nanotubes and did not observe a formation of large DOM flocks at the ionic strengths he was using; such an outcome would have complicated the interpretation.

Dr. Gordon commented that he has conducted experiments with nanoparticles and cod embryo and found similar low-dose effects, but not high-dose effects. Dr. Ferguson replied that he would like to do some of the same experiments at lower concentrations of SWNTs. He anticipated that he would see differences in aggregation behavior at different concentrations of the nanotubes.

Fate and Transformation of Carbon Nanomaterials in Water Treatment Processes
Jae-Hong Kim, Georgia Institute of Technology

The environmental impact of carbon fullerenes is of great concern because of projections for bulk production in the near future and the recent discovery that they form nanoscale water-stable aggregates upon release into the water. Understanding the fate and transformation of carbon fullerenes during water treatment, currently the first line of defense against ingestion pathways, is of particular importance. The objective of this research project is to examine the response of water-stable fullerene aggregates to processes used in potable water treatment, using C_{60} and its stable aggregate, nano-C_{60}, as a model compound. In the first 2 years of the project, researchers addressed the following questions: (1) How do carbon nanomaterials behave in a natural water matrix? (2) How does C_{60} react with chemicals used in water treatment? (3) How does C_{60} respond to UV irradiation with respect to the production of ROS? The researchers found that NOM enhances stabilization of carbon nanomaterials (i.e., C_{60}, SWNTs, and MWNTs) in natural waters. They also found that the adsorptive interaction between NOM and nanotubes depends on water quality parameters (e.g., pH and ionic strength) and NOM characteristics. Regarding the reaction of water-stable C_{60} aggregates with ozone, one of the strongest oxidants used during water treatment, the researchers found that the reaction products were molecular fullerene oxides and that the C_{60} cage structure remained intact in the product. Both mono- and di-oxygenated carbons were present, with hydroxyl and carbonyl functional groups. Further, the products showed pH-dependent UV spectra. Regarding the photochemical production of ROS by C_{60} in the aqueous phase during UV illumination, the researchers found that the status of C_{60} dispersion in the aqueous phase affects its ability to transfer absorbed photo-energy to oxygen. C_{60} present in water as stable aggregates did not produce singlet oxygen under UV illumination, in contrast to pristine C_{60}.

Discussion

Dr. Diallo pointed out that NOM generates superoxide. Dr. Kim replied that he corrected for that.

Dr. Lowry asked whether the surfactant might play a role in ROS production. Dr. Kim explained that, for energy transfer, the coating does not appear to have an effect. With superoxide, however, he did find an effect of surface coating on electron transfer and recombination.

Dr. David Barber asked whether the researchers have attempted similar experiments with the oxidized byproducts to examine ROS production. Dr. Kim answered that he has conducted such experiments and has observed singlet oxygen production.

Cross-Media Environmental Transport, Transformation, and Fate of Carbonaceous Nanomaterials
Peter Vikesland, Virginia Polytechnic Institute and State University

Little is known about the unintended health or environmental effects of manufactured nanomaterials, but some evidence suggests that they may be toxic. For example, nC_{60} produced using the THF method is suggested to cause oxidative stress in fish brain tissue and is potentially toxic to human cell lines. The goal of this research project is to examine carbonaceous nanomaterial fate and transport in the environment. In particular, the researchers determined how these particles behave when transferred between water and air. The project focuses on the characterization of the aqueous aggregates of C_{60} fullerene. The researchers found that aqueous nC_{60} particles (i.e., particles produced by extended mixing in water) are irregular in size and shape, have a negative surface charge, and are crystalline in nature. In contrast, THF nC_{60} particles are of regular size and shape, have a negative surface charge, and are crystalline in nature. Sodium citrate increases the negative surface charge of these particles at low concentrations but decreases the negative surface charge at higher concentrations. The researchers concluded that nC_{60} may form either via weathering of larger particles to smaller particles or recrystallization of nanoparticles from solution.

Discussion

Dr. Kim asked Dr. Vikesland to comment on the differences and similarities between the mechanisms of aggregate formation in the organic phase and the aqueous phase. Dr. Vikesland answered that he does not know how aggregates form in the organic phase or their characteristics.

Dr. Vicki Colvin asked Dr. Vikesland about the nature of the interaction with citrate or other stabilizers. She said that it is not clear that it would necessarily be ionic, and wondered how this interaction can be understood in a molecular sense. Dr. Vikesland responded that he would need to do Fourier transform infrared spectroscopy and other spectroscopic methods to characterize the nature of the interaction between citrate and the particle surfaces. He noted that researchers use acids to clean carbon nanotubes and to break the carbon cage; it is possible that, with extended mixing, the same process could occur with fullerenes, but this is not yet known.

Transport and Retention of Nanoscale C-60 Fullerene Aggregates in Water-Saturated Soils
Kurt Pennell, Georgia Institute of Technology

C_{60} forms stable nanoscale aggregates in water with aggregate diameters ranging from 95 to 200 nm depending on the preparation method and ionic strength. Only limited data are available on transport and retention of nC_{60} aggregates in porous media, and most previous studies employed high velocities and did not determine retention profiles. In addition, classical filtration theory has been used to describe nC_{60} transport and retention. The objectives of this research project are to: (1) investigate the transport and

retention of nC_{60} aggregates in water-saturated soils as a function of soil properties and systems parameters; (2) assess the effects of nC_{60} aggregates on soil water retention, water flow, and transport in unsaturated soils; and (3) develop and evaluate a numerical simulator(s) to describe nC_{60} aggregate transport, retention, and detachment in subsurface systems. The researchers found that nC_{60} aggregate transport decreases, and retention increases, as grain size or flow rate are decreased. The detachment rate coefficient approached zero and did not change with grain size or flow rate, indicating irreversible attachment. A mathematical model that includes nonequilibrium, nonlinear retention accurately captured nC_{60} transport and retention behavior in Ottawa sand. The researchers also found that ionic strength strongly influences nC_{60} aggregate transport and retention; the researchers attributed this primarily to electrostatic interactions. The secondary minimum plays an important role in nC_{60} attachment, and nC_{60} retention capacity was correlated with mass flux in the diffusional boundary layer. Future work will include: (1) measurement and simulation of nC_{60} transport and retention in water-saturated "natural" soil(s); (2) measurement and simulation of nC_{60} transport and retention in unsaturated porous media; (3) investigation of the effect of stabilizing or dispersive agents (e.g., NOM or surfactants) on nC_{60} transport and retention in Ottawa sands; and (4) determination of THF and γ-butyrolactone concentrations in purified and unpurified nC_{60} suspensions. In a separate project, the researchers will evaluate the neurotoxicity of manufactured nanomaterials.

Discussion

Dr. Saleh asked how the researchers separated fullerenes from the column. Dr. Pennell explained that the researchers sonicated and washed the column. They found that this worked best because they were primarily interested in finding mass balance.

Impacts of Fullerene (nC_{60} or C_{60}) on Microbiological Functions in Soil and Biosolids
Ronald Turco, Purdue University

Nanotechnology has tremendous potential for economic growth and is a key feature of sustainable development; however, almost nothing is known about the environmental impact of carbon-based manufactured nanoparticles. The goal of this research project is to provide fundamental information about the impact of C_{60} on the soil food web. This project addresses soils, biosolids, and fungi. The researchers found that C_{60} and nC_{60} had limited impact on the microbiology of soil and biosolids. In particular, soil biomass size and structure were unchanged. Repeated applications and solvent effects currently are under investigation. Biosolids biomass size and structure were unchanged by C_{60}. The researchers are investigating functional groups of C_{60} and carbon nanotubes in terms of their effects on the biomass of anaerobic digesters. Transformation of C_{60} by fungi also is limited and there is no evidence of fungal use of C_{60}. Studies of C_{60}-OH and fungi are ongoing and preliminary results are quite interesting.

Discussion

Ms. Lyon asked if the researchers sampled soil at time points earlier than 3 and 6 months and whether it is possible that there is a slight initial impact on bacteria, after which they recover. Dr. Turco responded that they sampled at 1 month and did not see any impact at that time point.

Size Distribution and Characteristics of Aerosol Released From Unrefined CNT Material
Judy Xiong, New York University School of Medicine

Particle concentration, size, distribution, shape, and agglomeration status are among the key factors for determination of worker exposure levels to airborne nanoparticles. Carbon nanotubes (CNTs) have a high aspect ratio, are highly agglomerated, and often coexist with other nanoparticles, such as amorphous carbon soot, metal catalysts, and ambient particulate matter. The size distributions of CNTs are difficult to

predict but presumably have a wide spread and are source-dependent. The specific aims of this study are as follows: (1) Investigation of the size distribution and characteristics of aerosol particles released from various types of industrial-grade CNT bulk materials resulting from agitation. The results will provide a foundation for developing field and personal sampling devices for CNTs. (2) Development of a practical method using atomic force microscopy (AFM) image analysis that is capable of classifying CNTs and distinguishing them from coexisting nano-sized particles in general environments. (3) Development of appropriate methods to monitor potential worker exposure levels to CNTs. The researchers found that all common types of unrefined CNTs, including single-walled, double-walled, and multi-walled nanotube samples, can be dispersed into the air to a significant extent from agitation. The sizes of particles generated from all CNT types were widely distributed across 13 stages of the Electrical Low-Pressure Impactor (ELPI), ranging from 7 nm to 10 µm. The size distributions varied with the type and the nature of bulk materials. For High Purity grade single-walled CNT produced by chemical vapor deposition, a majority of particles were in the nano-size region (< 100 nm) based on the ELPI data, and a significant proportion of particles also occurred in the single-nanometer range based on the data collected by an Integrated Screen Diffusion Battery (ISDB). Airborne CNT particles were highly agglomerated; no single tubes or simple ropes were observed by AFM in the original samples collected by ELPI or ISDB before treatment with surfactants. The researchers concluded that adequate monitoring methods should be established for quantification and characterization of these new types of materials to evaluate workers' exposure levels and the potential health risks. Ongoing studies include: (1) the development of a quantitative sample treatment method for AFM analysis that can effectively de-agglomerate samples by applying appropriate surfactants, solvent, and sonication; (2) an investigation of other advanced AFM technologies that may be better suited for CNT characterization, such as conductive-AFM and phase imaging; and (3) the development and validation of a field sampling method for airborne CNT particles in workplaces.

Discussion

Dr. O'Shaughnessy asked who manufactured the ISDB. Dr. Xiong answered that her research team made the ISDB in the previous research studies. Dr. O'Shaughnessy asked if the researchers based the ISDB on a particular source (Chang et al.), and Dr. Xiong said it was based on similar principles, but the ISDB was independently developed and designed at New York University. She added that the filtering elements of the ISDB could use either stainless steel wire screens of different mesh sizes or porous metal filters of different porosities. The researchers calibrated the collection efficiencies themselves.

Dr. Gordon asked whether it is common for researchers to collect data inside CNT manufacturing facilities or whether occupational monitoring programs exist anywhere. Dr. Xiong replied that she was uncertain about measurements conducted inside manufacturing facilities. She mentioned that some companies have developed workplace safety programs, but they lack appropriate sampling methods for CNTs. Dr. Savage added that NIOSH has been inside some of the CNT manufacturing facilities. She offered to find out more about this for workshop participants.

Physical and Chemical Determinants of Carbon Nanotube Toxicity
Robert Hurt, Brown University

It may be possible to reduce CNT health risks by understanding toxicity mechanisms and modifying the specific material features that trigger those mechanisms. The goal of this research project is to consider two characteristic nanotube features: catalytic impurities and hydrophobic surface area. The researchers found that all CNTs studied (both as-produced and "purified") release free metal (Fe, nickel [Ni], and yttrium) into physiological fluid phases; this triggers known toxicity pathways. Metal bioavailability is influenced by processing and environmental exposure. The researchers concluded that metal bioavailability assays should be standard in CNT characterization. The researchers also found that

SWNTs deplete essential micronutrients from the medium by physisorption and can affect cell behavior by a new *indirect* mechanism. α-Tocopherylpolyethyleneglycolsuccinate (TPGS), a water-soluble Vitamin E formulation, is a promising safe surfactant for the processing of carbon nanomaterials, especially MWNTs. In future work, the researchers will attempt to use TPGS to actively mitigate oxidant damage associated with nanomaterial exposure. Bioavailable metal in nanotubes probably can be removed by selective targeting as a simple detoxification strategy; this is the basis of pending future work.

Discussion

Dr. Saleh asked whether the researchers have systematically examined defects in CNTs and how that might affect absorption of amino acids and vitamins. Dr. Hurt responded that he and his colleagues had only limited data with which to address this question. They sulfonated the material; this introduces many hydrophilic sites, which suppresses absorption. He suggested that it is not really a question of active sites or defects, but rather the regions in between. With very defect-laden nanotubes, one can functionalize those defects and potentially suppress this problem. The more hydrophobic materials might adsorb more in solution.

Dr. Unrine noted that Ni metal particles can be difficult to solubulize in nitric acid even at high temperature and pressure. Considering that the researchers found sorption of cysteine and methionine, he asked whether they had considered how that might exacerbate metal toxicity and oxidative stress. Dr. Hurt said that he had not followed up on the biological implications of amino acid absorption, but the doses required to do that are high, well over 1 mg/mL. He noted that amino acid absorption may be relevant for some nanomedicine scenarios with high nanotube density; but for most nanotoxicity assays, vitamins will be absorbed but amino acids will not.

Environmental Impacts of Nanomaterials on Organisms and Ecosystems: Toxicity and Transport of Carbon-Based Nanomaterials Across Lipid Membranes
Dmitry Kopelevich, University of Florida

The hypothesis of this research project is that nanomaterials could lead to environmental dysfunctions because of the potential toxicity of these materials and their derivatives. In addition, their small size makes nanomaterials prone to biouptake and bioaccumulation, and their large surface area might allow them to act as carriers or deliverers of pollutants that are adsorbed onto them. The objectives of this project are to: (1) assess the toxicity of nanomaterials on biota using short-term microbiotests and investigate the impacts of nanomaterials on microbe-driven ecological functions; (2) determine the mobility of metal-based and carbonaceous nanomaterials in porous media, as well as the toxicity of nanomaterials in soil leachates; and (3) determine possible mechanisms of toxicity of different types of nanomaterials. In microbiotests, the researchers found that both C_{60} and SWNT toxicity significantly exceeded solvent toxicity. From biogeochemistry work, the researchers found that C_{60} toxicity significantly exceeded solvent toxicity and slowed down bacteria metabolism; these effects were sensitive to soil composition. It is not yet clear why SWNTs at small concentrations promoted algae growth. The researchers plan to investigate the effect of trace metals in SWNTs as opposed to the effects of the nanotubes themselves. They also plan to use the fluorescence of SWNTs to investigate the transport of SWNTs into cells and cell membranes; this will allow them to develop a connection with the molecular modeling studies. In the molecular modeling work, the researchers did not find a significant energy barrier for carbon-based particle penetration of the cell membrane's lipid bilayer. The particles had a long residence time inside the bilayer. Particle shape and size impacted the transport rates, dynamics, and localization of nanoparticles within the membrane. The researchers have collected preliminary data on physical effects on the membrane, such as the effect of particle size and shape. In future work, they will examine other potential physical effects on the membrane.

Discussion

Dr. Veronesi commented on the evidence for a possible physical deformation of the membrane and said that, from a neuroscientific perspective, several types of polymodal receptors respond to deformation, which initiates many types of inflammatory sequelae. Dr. Kopelevich suggested that the most interesting factor to examine would be the change in pressure profiles; he said that he hopes to have data to address this issue soon.

Dr. Hurt asked if the researchers are interested in looking at long-chain nanotubes. Dr. Kopelevich responded that he is interested in this but it would be computationally difficult.

Structure–Function Relationships for Predicting Nano-Bio Interactions
Vicki Colvin, Rice University

All stakeholders will benefit from an understanding of how fundamental characteristics of engineered nanoparticles control their biological effects. This research project will provide the first structure–function relationships for nanoparticle toxicology. The hypothesis of the research project is that nanoparticle structure (e.g., size and shape) directly controls cytotoxicity. A secondary hypothesis is that, of the four major material parameters in engineered nanoparticles (size, shape, composition, and surface), surface is the most important in governing cellular effects. The specific objectives are to: (1) expand the characterization of nanoparticle structure in biological media; and (2) characterize the effects of nanoparticles on cell function. The researchers found that the toxicity of nanoscale TiO_2 scales with photocatalytic activity and that the phase composition is crucial. They also found that QDs demonstrate minimal cytotoxicity at extracellular concentrations typical of most molecular imaging experiments (5–20 nm). The researchers confirmed in different QDs the previous finding that biocompatibility improves with PEGylation. They found that biocompatible polymer coatings appear to work by preventing cell uptake of nanoparticles.

Discussion

Dr. Hurt asked what the manufacturers of solar cell QDs would say is the potential for coatings that might make the material safer? Dr. Colvin replied that manufacturers probably will realize that they cannot build a business around QD systems unless they reclaim the Cd, and they are interested in packaging that allows for recycling to reclaim the Cd for further use. Some researchers are working on systems with the same efficiencies but without the use of heavy metals. This is promising, but the reality is that the companies manufacturing these materials are expanding in both their funding and the market. Dr. Hurt asked if polymer coatings are a serious option. Dr. Colvin responded that these coatings might not last in the environment after disposal. The systems should be packaged and encapsulated and, in general, the answer may be to recycle the materials and never allow them to enter a landfill.

A participant asked if the researchers had any information on how the QD coating might affect their photoactivity. Dr. Colvin replied that she and her colleagues are making the QDs as they are because the QD is already essentially stabilized by an organic substance and the chemistry that produces it. When this coating is stripped, the photoactivity of the material is destroyed. The organic coatings must be left intact or the material will not be effective for solar cells or other applications. She noted that her coatings do not perturb the native coatings of the QDs; the researchers encapsulate, rather than strip, the QDs. They examined the kinds of highly photoactive substances that researchers in biomedical engineering or in solar cell work would want to use.

Cellular Uptake and Toxicity of Dendritic Nanomaterials
Mamadou Diallo, California Institute of Technology

The overall objective of this research project is to improve understanding of the cellular uptake and toxicity of dendritic nanomaterials in aqueous solutions at physiological pH 7.4. The specific objectives are to: (1) characterize the interactions of dendrimers with cell membranes through measurements of physical–chemical surrogates (octanol–water partition coefficients and liposome–water partition coefficients); (2) characterize the interactions of dendrimers with plasma proteins through measurements of dendrimer binding to human serum albumin (HSA) protein; (3) use molecular dynamics simulations, nuclear magnetic resonance spectroscopy, neutron scattering, and neutron reflectometry to characterize the mechanisms of interactions of dendrimers with lipid bilayers and HSA protein; (4) characterize the cytotoxicity of dendrimers through *in vitro* measurements of cell viability and toxicogenomic studies; and (5) conduct a correlation analysis. Work in progress includes examination of the mode of cell death, the mechanisms of dendrimer cytotoxicity, and cellular uptake and subsequent activation of intracellular signal transduction pathways. The successful completion of this project should provide industry with critical data and predictive tools needed to assess the health and environmental impact of dendritic nanomaterials, such as ethylene diamine core poly(amidoamine) dendrimers.

Discussion

Dr. Veronesi asked if surface charge affects transcytosis through cells. Dr. Diallo responded that surface charge does affect this. He noted that he and colleagues will first use biological imaging and then will conduct toxicogenomics work to determine the mechanism.

Interactions of Pure and Hybrid Polymer Nanofibers With Cells
Perena Gouma, State University of New York at Stony Brook

Nanostructured materials offer a high surface area-to-volume ratio and interconnected porosity. They are used in tissue engineering to produce fibrous scaffolds that mimic the extracellular matrix (ECM). This research project focuses on natural polymer–hydroxyapatite nanofiber interactions with osteoblasts, which are anchorage-dependent, mononucleate cells responsible for bone formation. Cellulose acetate (CA) is a natural polymer that has been used as a scaffold material for functional cardiac cells and microvascular cell growth. Hydroxyapatite (HA) is a bioactive material that promotes osteoblastic differentiation *in vitro*. HA is used in bone tissue engineering. Nanocrystalline HA is similar to bone apatite. Adding nano-HA to natural polymer hybrids is expected to strengthen cell–polymer fiber interactions. The researchers studied the interactions of human osteoblasts with fibrous nanomaterials and their hybrids. They used electrospinning to fabricate the nanofibrous mats for bioscaffolds. The researchers found that the osteoblasts maintained a rounded morphology on CA fibers and typically attached to a single fiber. In contrast, osteoblasts tended to remain flat on CA–HA nanofibers and attached to several fibers, forming an interconnected network. The osteoblasts also preferentially attached to thinner fibers. HA nanoclusters provided anchoring sites for osteoblasts, thus enhancing cell attachment. Further, the CA–HA hybrid mats showed enhanced cell spreading, which is known to control cell differentiation. The researchers concluded that the size and shape of nanomaterials play important roles in influencing cell attachment and proliferation behavior.

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Announcements
Warren Layne, U.S. EPA

Dr. Layne announced an international nanotechnology meeting scheduled to take place October 6–8, 2008, in Chicago. EPA currently is compiling a list of invited speakers. Dr. Layne noted that information about this meeting will be available on the EPA Web Site soon.

Assessment Methods for Nanoparticles in the Workplace
Patrick O'Shaughnessy, The University of Iowa

A typical industrial hygiene analysis of workplace dust exposure does not include instrumentation to detect particles in the nanometer size range. The objectives of the research project were to: (1) identify and evaluate methods to measure airborne nanoparticle concentrations; (2) characterize nanoparticles to assess their surface and bulk physical and chemical properties; and (3) determine the collection efficiencies of commonly used respirator filters when challenged with nanoparticles. The researchers compared a surface area analyzer, handheld condensation particle counter (CPC), photometer, electrostatic precipitator, scanning mobility particle sizer, and an optical particle counter (OPC). They analyzed Fe oxides at high and medium concentrations; TiO_2 at high, medium, and low concentrations; and carbon nanotubes. The results indicate a need to apply a shape factor to make direct correlations between instruments, especially when comparing among instruments with different units, such as count, surface area, or mass concentrations. This information will be useful for comparing results obtained by different instruments and for choosing an appropriate instrument for evaluation of nanoparticles in the workplace. In field sampling at a nanostructured lithium titanate facility, the researchers found that material handling of lithium titanate dispersed this material as large particles (> 1 µm); any nano-sized particles observed were mainly associated with other sources, such as diesel forklifts and welding and grinding operations.

Discussion

Dr. Savage asked about the instruments the researchers used to do the facility measurement for the field sampling. Dr. O'Shaughnessy replied that they primarily used the handheld CPC and the OPC. They compared the results to gravimetric measurements using more traditional methods and the photometer.

Dr. Layne asked how uniform the agglomerates are in size. Dr. O'Shaughnessy responded that the particles produced in his laboratory are between 90 and 150 nm, but this size distribution is partly an artifact of the methods used to produce the particles. He referenced Dr. Gordon's work and suggested that aging might allow more time for agglomeration to occur. In terms of results from field sampling, however, the size distribution is still uncertain. What remains unknown is the particle sizes to which people are actually exposed.

Mr. James Stewart noted that asbestos analysis is a parallel to the TEM particle counting process. He asked if the researchers have looked into that kind of analysis for particle counts. Dr. O'Shaughnessy replied that there is a NIOSH method for TEM of asbestos and the researchers will be looking into this method. Asbestos particles, however, are larger and better defined than the agglomerates under study in this project.

Development of Nanosensors for the Detection of Paralytic Shellfish Toxins
Robert Gawley, University of Arkansas

The goal of this research project is to develop nanosensors for the detection of paralytic shellfish toxins (PSTs), primarily saxitoxin. (Other shellfish toxins are chemically very similar to saxitoxin.) Currently, the primary methods for detection of shellfish toxins are the mouse bioassay and the Lawrence High-Performance Liquid Chromatography Method; each of these methods has limitations. The researchers have been developing another method—fluorescence sensing, which is based on the 1:1 equilibrium between the toxin and the sensor to produce a fluorescent complex; the sensor in the absence of the toxin is not fluorescent. The sensors are based on crown ethers. Using a new class of crown ethers, the researchers are achieving 100% fluorescence enhancement with a 1 μM concentration of saxitoxin. More recently, the researchers have investigated sensing in a nanoscale self-assembled monolayer with a long-term aim of placing the sensor in a portable device. They have been attempting to determine which chromophore will work best. Scientists at the Department of Health in Seattle provided the researchers with shellfish extracts (specifically, blue mussel extracts); this helped them determine the limitations of each of the chromophores. A method not related to fluorescence that currently is under investigation uses the protein saxiphilin, which was isolated originally from bullfrogs. The C-lobe of saxiphilin binds saxitoxin with nanomolar affinity. The researchers are attempting to use this protein in the development of an electrochemical displacement assay. They hope to place the C-lobe of saxiphilin in a monolayer in a microfluidic device for PST detection.

Discussion

Dr. Wiggins asked if the researchers are using a dichroic filter system to detect fluorescence when they use fluorophores to create a fluorescence wavelength. Dr. Gawley confirmed that this was correct. Dr. Wiggins asked if it would be possible to achieve a more sensitive level of detection (despite some loss of efficiency) by doing away with the dichroic filter, polarizing the fluorescent light, and using fluorescent depolarization to detect the fluorescence. Dr. Gawley agreed that this would be possible. He and his colleagues primarily are working with a fluorometer rather than a microscope and have been working with another company to optimize this kind of response.

Dr. Layne asked whether the crown ether materials are easy to synthesize. Dr. Gawley responded that the parent crown ether can be purchased with one or two nitrogens, and one then may alkylate one or both of the nitrogens. Synthetically, the process is trivial.

Bioavailability and Fates of CdSe QDs and TiO_2 Nanoparticles in Bacteria
Patricia Holden, University of California at Santa Barbara

The goal of this research project is to investigate the influence of bacteria on nanoparticles and the influence of nanoparticles on bacteria. The specific objectives are to: (1) quantify planktonic bacterial toxicity to and uptake of cadmium selenide (CdSe) QDs; (2) investigate interactions between nanoparticles and bacterial biofilms; (3) investigate redox mechanisms with QDs; and (4) investigate size-related toxicity of TiO_2 nanoparticles. The researchers found that, in the presence of bare, 5-nm CdSe QDs, planktonic *Pseudomonas aeruginosa* growth was inhibited in a dose-dependent manner as it was in the presence of cadmium ion [Cd(II)] at equivalent concentrations. The bacteria accumulated Cd in cells whether they were fed CdSe QDs or CdII. They also found that planktonic *P. aeruginosa* broke down CdSe QDs and this breakdown appeared to be cell-associated. It is not yet known whether one can predict toxicity of a heavy metal-containing nanoparticle based on its heavy metal content. In biofilm *P. aeruginosa*, the researchers found that the toxicity profiles of Cd were similar to those for planktonic *P. aeruginosa*. Accumulation of Cd was similar for biofilm and planktonic *P. aeruginosa*, except that in the biofilm bacteria, Cd also accumulated in the extracellular polymers, both in QD- and Cd(II)-exposed

cells; it is not clear if the Cd in the extracellular polymers is in the form of CdSe or Cd(II). The researchers observed a Cd concentration gradient in the medium, with lower concentrations between biofilms and concentrations that were 100-fold higher in or under the biofilms. It is not yet known if the mechanism of Cd toxicity is similar for planktonic and biofilm bacteria. The researchers investigated the potential for electron transfer in the interactions between bacteria and nanoparticles using time-correlated single proton counting and measuring lifetime fluorescence emission of dopamine (DA)-conjugated QDs. The researchers found that lifetime fluorescence was enhanced for concentrated *Escherichia coli* associated with QD-DA compared to either QD-DA alone or diluted *E. coli* associated with QD-DA. It is not yet known if interfacial charge transfer occurs from cells to QDs. To address the fourth objective, the researchers are working with the bacterium *P. putida* and several types and diameters of TiO_2 particles. They found that *P. putida* growth decreased in the presence of TiO_2; specifically, the larger TiO_2 particles appear to be toxic and the effects are dose-dependent. Further, they found that TiO_2 aggregates appeared to break down during bacterial growth, implying that the bacteria break down the aggregates. In the future, the researchers will examine the greater toxicity of larger TiO_2 particles. They also hope to conduct a systematic study to determine how the bacteria are breaking down the aggregates.

Discussion

Dr. Barbara Walton asked if Dr. Holden could envision that either the aggregation or breakdown of TiO_2 could be related to the differences between rutile and anatase crystal structures. Dr. Holden replied that she thought that was possible. She and her colleagues have not yet tested this, but they would be very interested in investigating this possibility.

Dr. Gawley asked whether the kind of de-aggregation shown with TiO_2 has been done with CdSe. Dr. Holden clarified that the researchers have not observed aggregation of the CdSe QDs with which they work; they start with dispersed particles that remain dispersed. In the case of QDs, the nanoparticles themselves are breaking down and liberating the Cd(II); the cells are facilitating the breakdown. She did not know whether aggregated CdSe QDs would be broken down by bacteria but agreed that this would be interesting to test.

Dr. Yongshen Chen asked if the bacteria must internalize the QDs to degrade them. Dr. Holden noted that she had been very careful not to say that QDs are broken down *inside* cells; she can only say that the degradation is cell-associated. It is not yet clear if the degradation is happening inside the cell or at the surface of the cell. Similarly, with biofilms, the researchers do not yet know the form of the Cd. Dr. Chen asked about the addition of azide and Dr. Holden clarified that this was part of the experiment with *E. coli* regarding electron transfer. Dr. Chen asked if cell metabolism was inhibited (or if the cells were killed). Dr. Holden responded that she and her colleagues are planning to investigate this issue.

Dr. Veronesi asked if QDs shift the absorbance of DA when oxidized or reduced. Dr. Holden answered that this was correct and, in the oxidizing conditions, the emissions observed are primarily from the DA breakdown product. In the case of reducing conditions, the emissions are from the QD itself because the DA is lost.

A Novel Approach to Prevent Biocide Leaching
Patricia Heiden, Michigan Technological University

The objective of this research project is to develop a practical and effective approach to prepare controlled-release, biocide-loaded nanoparticles that can be efficiently introduced into wood to reduce or eliminate biocide leach into sensitive environments (e.g., wetlands). Preventing biocide loss to leach also should extend the lifetime of treated wood products. The researchers have adapted a method to prepare core-shell nanoparticles, each with a hydrophobic core that serves as a biocide reservoir and moderates

the biocide release rate. The main research components include: (1) development of the nanoparticle method; (2) development of efficient wood treatment; and (3) assessment of results to determine how effectively the nanoparticles are reducing biocide leach. The researchers have been able to prepare nanoparticles with a dry diameter of approximately 40 nm, which is smaller than the target diameter (no more than 150 nm); however, in water the gelatin shell swells, increasing the diameter to 150–300 nm, and it is not clear if this swelling is problematic for this application. The nanoparticle yield appears to be sufficient, but when isolated, only 45–55% dry nanoparticles are collected; the target yield is greater than 90%. The researchers have achieved approximately 46 wt% incorporation of biocide, compared with a target of 48 wt%. Work is ongoing to improve the method and to identify the optimal core:shell (hydrophobic:hydrophilic) ratio to balance biological efficacy and biocide leach. In terms of wood treatment, the researchers have found that delivery efficiency appears to be 84–97% (compared to a target of greater than 90%) and have demonstrated biological efficacy. Preliminary results also show a significant decrease in biocide leach in nanoparticle-treated wood.

Discussion

Dr. Wiggins asked what the hydrophobic core was in this research project. Dr. Heiden responded that it was methyl methacrylate. Dr. Wiggins wondered if the researchers had considered the option of making the nanoparticles by mixing the biocide with phospholipids; this would form a hydrated shell. One could make this onsite very simply by adding water. Dr. Heiden replied that industry has used surfactants with the organic biocides, but this method also is very susceptible to leach.

Evaluating the Impacts of Nanomanufacturing via Thermodynamic and Life Cycle Analysis
Bhavik Bakshi, The Ohio State University

The overall goal of this research project is to help guide the development of nanotechnology so it is environmentally benign and sustainable. Understanding the impact of nanomaterials is essential, but this is not sufficient; it is necessary to adopt a systems view with life cycle thinking. LCA of emerging technologies poses unique challenges. In particular, life cycle inventory data for nanomanufacturing are not available and the impacts of engineered nanomaterials on humans and ecosystems are only partially known. Predicting life cycle processes and activities is difficult because the technology is still in its infancy. The first objective of this research project is to conduct a life cycle evaluation of nanoproducts and processes. In particular, the researchers will establish life cycle inventory modules for nanomaterials and perform an LCA of polymer nanocomposite products. The second objective is to explore a predictive model for LCA and impact assessment. Specifically, the researchers will examine the relationship between life cycle inputs and impact and the relationship between the properties of nanoparticles and their impacts. The researchers have found that, on an equal mass basis, carbon nanofibers require a significantly higher energy investment, and appear to have a larger life cycle environmental impact, than traditional basic materials. The high energy investment may lead to high costs, thus restricting the use of carbon nanofibers to niche applications. Products based on carbon nanofibers may be greener than alternatives for certain applications, and the quantity used will be the deciding factor. Regarding the predictive model, the researchers found that, for emerging technologies, input information is easier to obtain than output information. Preliminary results indicate a promising correlation between life cycle inputs and impact. They found that ecological cumulative exergy consumption appears to be best for aggregating inputs for a predictive LCA. The relationship between toxicology of nanoparticles and thermodynamic properties also is promising. Future work will include: (1) an LCA of conventional versus nanocomposite materials; (2) further statistical evaluation of the relationship between inputs and impact; (3) an exploration of the relationship between thermodynamic properties of nanoparticles and their toxicity; and (4) risk analysis.

Discussion

Dr. Unrine commented on the mass-to-mass comparison and the application to automotive parts. He suggested, for example, that if automobile body panels were made from carbon fiber, the car would be lighter than one made with steel or aluminum and its operation would require less energy. He asked if the model could take this into account or if the researchers had any data to address this issue. Dr. Bakshi agreed that the mass of carbon nanofibers in automobile body panels would be small. The researchers currently are examining nanocomposite materials. He cautioned, however, that most engineers and scientists tend to think that if they develop a technology that uses less material, uses less energy, and is more efficient, this will be better from an environmental perspective. Unfortunately, this does not always happen because a technology that is more efficient also is less expensive, so it tends to be used more extensively. Therefore, if the nanotechnology of carbon nanofibers became very good, they would likely be used in almost everything and would be used much more extensively. In this way, any advantage may disappear.

Dr. Mayer noticed that most of Dr. Bakshi's work is based on energy consumption and it appears that energy consumption currently is not well understood. He asked if Dr. Bakshi believed that, in future years, the data regarding energy consumption will be tighter, resulting in better correlations. Dr. Bakshi explained that the researchers focused on energy consumption because this was the easiest approach, but he clarified that he and his colleagues also had examined emissions of many other chemicals and consumption of other materials. Regarding the large error bars for some analyses presented, he said that he is in the process of completing a calculation of the theoretical minimum amount of energy needed to produce carbon nanofibers; the challenge for this calculation is that many of the properties of nanomaterials are not very well known. With this calculation, the error bars will no longer be relevant. He added that the error bars in the analyses presented are based on the cycle time; according to manufacturers the cycle time can be quite variable and this is why the error bars are so large.

Dr. Saleem remarked that EPA has a 2003 database for the TRACI Model and the LCA Model. Dr. Bakshi explained that, even though the EPA data are more current, the underlying model is based on information from the Bureau of Economic Analysis, and 1997 is the most recent database available. The 2002 model will come out next month and the researchers will incorporate the 2002 data.

Dr. Philip Lippel observed that it seems, generally, that one of the big promises of nanomaterials is the potential to use much less material for the same purpose. He guessed that one might expect a reduction of a factor of 10 or even a factor of 100 for most applications. This may be application-specific, but he expected that a correction factor may be needed in the mass comparison. In addition, as these materials become more widely used, the researchers may find that they are comparing immature production technologies to mature ones. He asked Dr. Bakshi to what extent he expects the energy production costs for carbon nanofibers to decrease. He added that Southwest Nanotubes is one company that may be willing to provide relevant data. This company has been communicating very publicly recently about scaling up through three or four generations of continued flow reactor designs and it might be willing to share information about the associated energy costs. Such information might be helpful to the researchers' modeling efforts. Dr. Bakshi replied that he will contact that company. He also described some of the ways in which he and his colleagues are addressing this kind of concern. For example, the researchers will determine theoretically the minimum amount of energy needed. In addition, they will specifically examine the computer chip industry to determine how energy consumption has declined historically. They then will conduct a scenario analysis based on this information. This work is ongoing and Dr. Bakshi said he expects to have results next year.

Dr. Savage thanked all the participants for their contributions and adjourned the meeting.