

# Abstract Book

ICEIN 2009

September 9-11, 2009

Howard University, Washington, DC



**ICEIN 2009, Blackburn Center, Howard University, Washington, DC**

**Wednesday, 9 September 2009**

9:00-9:05	<b>Introduction/Welcome, West Ballroom</b>		
9:05-9:15	Kimberly Jones, Howard University		
9:15-9:25	Judith Verbeke, NSF Nora Savage, EPA		
9:25-9:55	<b>Presentation of CEINs</b>		
9:55-10:25	Mark Wiesner, CEINT Andre Nel, UC CEIN		
10:25-10:40	Break		
10:40-11:20	Session 1: West Ballroom <b>Fate, Transport &amp; Transformation</b> Co-Chairs: Mark Wiesner/Sharon Walker <b>Keynote: Greg Lowry/Arturo Keller</b> , Overview of the Challenges in Predicting NP Fate and Transport	Session 2: Forum 144/146 <b>Toxicity/ Ecotoxicology</b> Co-Chairs: Richard Di Giulio/Patricia Holden <b>Keynote: Anne Kahru</b> , From Ecotoxicology to nanoecotoxicology	Session 3: Auditorium 151/155 <b>Risk Perception, Risk Assessment &amp; Life Cycle Assessment</b> Co-Chairs: Elizabeth Casman/Hillary Godwin <b>Keynote: Igor Linkov</b> , Linking Risk Assessment, Decision Analysis and Life Cycle Assessment for Guiding Nanomaterials Development
11:20-11:40	<b>Eric M.V. Hoek</b> , Influence of Water Chemistry on the Stability and Toxicity of Metal and Metal Oxide Nanoparticles	<b>Andre Nel</b> , Using Predictive Toxicological Paradigm to Study Nanotoxicity and Design Safe Nanomaterials	<b>Christine Robichaud</b> , Estimating Nanomaterial Emission Sources from Production Information
11:40-12:00	<b>Armand Masion</b> , Aging of Commercial Nanomaterials: a New Environmental Challenge?	<b>Joel A. Pedersen</b> , Developmental Toxicity of Oxidatively Degraded Quantum Dots	<b>Eric Money</b> , Nano-Risk and Macro-Uncertainty: Using Probability Networks to Model the Environmental Implications of Nanotechnology
12:00-2:00	Lunch		
2:00-2:20	Session 1 cont. <b>Tanapon Phenrat</b> , Semi-Empirical Correlation to Predict the Collision Efficiency of Natural Organic Matter (NOM) - and Polymer-Coated Nanoparticle	Session 2 cont. <b>Cole Matson</b> , Silver Nanoparticle Behavior and Fish Embryotoxicity across a Salinity Gradient	Session 3 cont. <b>Melanie Auffan</b> , What Nanoparticles Merit Special Considerations from an Environmental, Health, and Safety Perspective?
2:20-2:40	<b>Robert Hamers</b> , Thermal and Oxidative Stability of Ligand-Modified Nanoparticles: Implications for Environmental Safety of Nanoparticles	<b>Kevin Kwok</b> , Implications of Silver Nanoparticles for Eleutheroembryos and Larval Medaka ( <i>Oryzias latipes</i> )	<b>Milind Kandlikar</b> , The Impact of Toxicity Testing Costs on Nanomaterial Regulation
2:40-3:00	<b>Ernest Hotze</b> , Structure Affecting Reactivity: The Fullerene Example	<b>Elise Fairbairn</b> , Effects of Metal Oxide Nanomaterials on Sea Urchin Embryo Development	<b>William Freudenberg</b> , Real Risk, Perceived Risk, and Nanotechnology: Lessons from Current Research and Past Environmental Controversies
3:00-3:20	<b>Jorge L. Gardea-Torresdey</b> , CeO <sub>2</sub> and ZnO Nanoparticles Undergo Differential Biological Transformations in Soybean Plants	<b>Joel Meyer</b> , Silver Nanoparticles Inhibit Growth in <i>Caenorhabditis elegans</i>	<b>Darin Furgeson</b> , Nanotoxicity Assessments with a Whole Zebrafish Model
3:20-3:40	Break	Break	Break
3:40-4:00	<b>Matthew Hull</b> , Effects of Surface Coating and Solution Ionic Composition on Aggregation of Au Nanoparticles in Freshwater Systems	<b>Emilie Bigorgne</b> , Ecotoxicity of Altered TiO <sub>2</sub> Nanocomposite on the Earthworm, <i>Eisenia fetida</i>	
4:00-4:20	<b>Sharon Walker</b> , Transport of TiO <sub>2</sub> Nanoparticles: Role of Solution Chemistry and Particle Concentration	<b>Camille Larue</b> , Investigation of nanoparticles and carbon nanotubes toxicity and transfer in plants	
4:20-4:40	<b>Andrea Tiwari</b> , Oxidation of Carbonaceous Nanoparticles in the Atmosphere: Effects of Ozonation on C <sub>60</sub> Aqueous Solubility	<b>Cyndee Gruden</b> , Impact of Metal Oxide Nanoparticles on Microbial Cell Viability	
4:40-5:00	Break		
5:00-7:00	<b>Poster Session</b>		

**Thursday, 10 September 2009**

9:00-9:40	Session 4: Room 148/150 <b>Natural Nanomaterials &amp; Nanobiogeochemistry</b> Co-Chairs: Michael Hochella, Jr./Jorge Gardea-Torresdey <b>Keynote: Mitchel Doktycz</b> , Microbially Mediated Transformation of Metal and Metal Oxide Nanoparticles	Session 5: Forum 144/146 <b>Nanomaterial Characterization &amp; Toxicity Screening</b> Co-Chairs: Jie Liu/Ponisseril Somasundaran <b>Keynote: Martin Hassellöw</b> , Challenges and Current State of Physicochemical Characterization and Detection of Nanoparticles in Environmental Media	Session 6: Auditorium 151/155 <b>Ecology &amp; Ecosystem Response</b> Co-Chairs: Paul Bertsch/Bradley Cardinale <b>Keynote: Emily Bernhardt/Joshua Schimel</b> , Known Knowns, Unknown Knowns and Unknown Unknowns: Challenges to Understanding the Impacts of Nanomaterials in Ecosystems
9:40-10:00	<b>Michael Hochella, Jr.</b> , Nanominerals, Mineral Nanoparticles, and Earth Systems: A Partial but Useful Guide to the Behavior of Anthropogenic Nanomaterial	<b>James Mitchell</b> , Analytical Characterization of Silver Nanoparticles and Nanoparticle Doped Media	<b>Robert J. Miller</b> , Differential Effects of Nano-Particulate Zinc Oxide and Titanium Dioxide on the Population Growth Rates of Marine Phytoplankton
10:00-10:20	<b>Galen D. Stucky</b> , Synthesis, Size-Selective Separation, and Chemistry of Composite Synthetic Natural Nanoparticles	<b>Saji George</b> , High Throughput Multiparametric Cytotoxicity Screening for Predictive Toxicological Assessment of Nanomaterials	<b>Christina Arnaout</b> , Microbial Inhibition and Silver Resistance Development in Wastewater Containing Silver Nanoparticles
10:20-10:40	<b>Gordon Brown</b> , Molecular-Level Studies of Iron Oxide and Silver Nanoparticles - Structure and Reactivity	<b>Lee Ferguson</b> , Analysis of Single-Walled Carbon Nanotubes in Estuarine Sediments by Near Infrared Fluorescence Spectroscopy	<b>Bradley Cardinale</b> , TiO <sub>2</sub> Nanoparticles Stimulate Biomass Production in Freshwater Algae
10:40-11:00	Break	Break	Break
11:00-11:20	<b>Dongxu Zhou</b> , Stability and Photoactivity of Metal Oxide Nanoparticles in Natural Waters	<b>Yingwen Cheng</b> , Stability of Silver Nanoparticles Under the Irradiation of Sunlight	<b>Teresa Kirschling</b> , the Impact of Reactive Iron Nanoparticles on Microbial Diversity in Aquifer Soils
11:20-11:40	<b>Andreas Gondikas</b> , Surface Interactions of Thiol-Containing Organic Acids on Zinc Sulfide and Silver Nanoparticles	<b>Xiaohua Fang</b> , Correlation of Wettability/Hydrophobicity of Nanoparticles with their Toxicity on <i>Nitrosomonas europaea</i>	<b>Antoine Thill</b> , Physicochemical and Biological Interactions Between Manufactured Nanoparticles and Environmental Bacteria
11:40-12:00	<b>Rebecca French</b> , Schwertmannite: A Natural Nanomaterial's Role in Arsenic Cycling	<b>Zhaoxia (Ivy) Ji</b> , Optimization of Nanoparticle Dispersion in Various Cell Culture Media	<b>Jason Unrine</b> , Analytical Approaches for In Situ Characterization of Metal and Metal Oxide Nanoparticles in Soil Ecosystems
12:00-12:20		<b>Elizabeth Suarez</b> , UCLA Molecular Shared Screening Resource: Capabilities and Applications for Nanotoxicity Studies	<b>Randy Mielke</b> , Bioaccumulation and Biomagnification of CdSe Quantum Dots in a Simplified Microbial Food Chain
12:20-2:00	Lunch		
2:00-2:30	<b>Hilary Godwin</b> , Engaging and Effectively Communicating our Results to Broad Range of Stakeholders: Education and Outreach Activities in the UC CEIN		
2:30-3:00	<b>Glenda Kelly</b> , Education and Outreach Initiatives in CEINT: Targeted Communication of Results Across Diversified Audiences		
3:00-3:45	<b>International Efforts for Protocols Development</b>		
3:45-4:00	<b>C. Michael Garner</b> , IANH Protocols Development		
4:00-5:00	Break	Break	Break
4:00-5:00	Panel 1: Room 148/150 <b>Protocols - Material Characterization &amp; Preparation</b> Co-Chairs: Mark Wiesner/Arturo Keller	Panel 2: Forum 144/146 <b>Protocols - In Vivo Testing</b> Co-Chairs: Cole Matson/Gary Cherr	Panel 3: Auditorium 151/155 <b>Protocols - In Vitro Testing</b> Co-Chairs: Claudia Gunsch/Patricia Holden

**Friday, 11 September 2009**

9:00-10:30	Committee 1: Room 148/150 <b>Nanomaterial Availability &amp; Standards</b> Co-Chairs: Jie Liu/Jeffrey Zink	Committee 2: Forum 144/146 <b>Development of New Methods for Measurement in Complex Media</b> Co-Chairs: Lee Ferguson/Jorge Gardea-Torresdey	Committee 3: Auditorium 151/155 <b>Ecosystem Discussion</b> Co-Chairs: Emily Bernhardt/Hunter Lenihan
10:30-10:45	Break		
10:45-11:45	<b>Report Back from Panel and Committee Discussions, and Adjournment, Auditorium 151/155</b>		

Session 1: Fate, Transport & Transformation

Co-Chairs: Mark Wiesner and Sharon Walker

Wednesday, September 9, 2009

10:40 a.m. – 4:40 p.m.

West Ballroom

# **Overview of the Challenges in Predicting NP Fate and Transport**

**Greg Lowry<sup>1</sup> and Arturo Keller<sup>2</sup>**

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<sup>2</sup>UC Santa Barbara, Santa Barbara, CA.

The ability to predict the transport, transformations, and fate of nanomaterials released to the environment is paramount to assessing the potential for exposure and adverse effects to the environment and to human health. There are many challenges characterizing the types and rates of transformations that may occur in the environment given the variety of environmental conditions, e.g. pH, redox state, moisture content, microbial communities, sunlight intensity, salinity, and availability of labile organic carbon. Each will affect the types and rates of environmental transformations and mobility of nanomaterials released to the environment. Adsorption of organic macromolecular surface coatings, or those initially present on the nanoparticle will also strongly affect transport and transformations of nanomaterials, and their interactions with biota. We will present an overview of the research activities on UC-CEIN and CEINT aimed at characterizing the fundamental processes affecting the fate, transport, and transformations of nanomaterials in environmental matrices, and how these transformations influence bioavailability and persistence of those materials. Processes of interest include 1) aggregation/agglomeration, 2) nanoparticle-macromolecule interactions, 3) nanomaterial-biota interactions and bioavailability of nanoparticle surface coatings, 4) dissolution/disaggregation, and 5) oxidation and reduction reactions.

# **Influence of Water Chemistry on the Stability and Toxicity of Metal and Metal Oxide Nanoparticles**

X. Jin<sup>1,3</sup>, C. Marambio-Jones<sup>1,3</sup>, E.M.V. Hoek<sup>1,3,\*</sup>, R. Damoiseaux<sup>2,3</sup>

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<sup>3</sup> UC Center for the Environmental Implications of Nanotechnology (UC CEIN), Los Angeles, California, USA

\* presenter

In most available studies, cytotoxicity of nanoparticles is evaluated in nutrient rich culture media or on culture plates. However, nanoparticles assume variable aggregation states in different aquatic media because the ionic strength, electrolyte composition, organic content and redox state can be radically different from that of laboratory growth media. Therefore, nanoparticle stability, toxicological mechanisms and toxicity are sure to vary in different aquatic media. In this study, the physicochemical properties, aggregation state, and antibacterial properties of metal and metal oxide nanoparticles were evaluated in real river water, wastewater effluent, and ocean water as well as laboratory prepared aquatic media designed to model their ionic and organic contents.

As part of the UC CEIN, a small library of metal and metal oxide nanoparticles of (commercial and laboratory origins) was studied. Simulated aquatic media included three combinatorial libraries of cations, anions, and dissolved organics designed to represent the freshwater, wastewater, and seawater. High throughput bacteriocidal and bacteriostatic assays were developed to assess bacterial toxicity. Extensive physical-chemical characterizations (light scattering, electrophoretic mobility, electron microscopy, X-ray spectroscopy) were performed to gain further insight about the relationships between water chemistry, nanoparticle aggregation-dissolution state, and bacterial toxicity.

Generally, the characteristic sizes of nanoparticles in aquatic media are much larger than the vendor-reported sizes of primary particles, but in many water chemistries primary particles were well dispersed and, in some cases, undergoing rapid dissolution. Light scattering and electron microscopy confirm widely varying aggregation states in the different water matrices. Bacteriocidal assay data show that low concentrations of nanoparticles cause some cell death; however, bacterial inactivation was different in each water matrix. Bacteriostatic assay results show that laboratory prepared aquatic media produce oligotrophic conditions, and hence, only high concentrations significantly inhibit the already low growth rates. We will discuss the emerging relationships between intrinsic nanoparticle properties, water chemistry (e.g., differences in salinity, hardness, alkalinity, dissolved organics), aggregation-dissolution state of nanoparticles, and bacterial toxicity of metal and metal oxide nanoparticles.

# **Aging of commercial nanomaterials: a new environmental challenge ?**

Armand Masion<sup>1</sup>, Céline BOTTA<sup>1</sup>, Jérôme ROSE<sup>1</sup>, Jérôme LABILLE<sup>1</sup>, Mélanie AUFFAN<sup>2</sup>, Perrine CHAURAND<sup>1</sup>, Jeanne GARRIC<sup>3</sup>, Jean-Yves BOTTERO<sup>1</sup>

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With more than 800 manufacturer-identified nanotechnology-based consumer products currently on the market, nanomaterials have become part of our daily life. They are found in electronics, cars, cosmetics, medical products... While sales of these products are increasing, the question about the life cycle of nanomaterials remains largely unanswered: possible degradation and release as well as the fate/disposal of nanomaterials after their intended use are critical points that need to be addressed. While some nanomaterials are designed to last, others are clearly intended for a short-term use. Here we will focus on products of this second category, viz. sunscreens, whose UV filtering properties are due to nanosized TiO<sub>2</sub> with a double coating of AlOOH and polydimethylsiloxane (PDMS). This nanomaterial was aged in water under several physical-chemical and UV irradiation conditions. The double coated TiO<sub>2</sub> nanocomposite progressively acquired a hydrophilic character, thus indicating the degradation of the organic outer coating. This degradation was confirmed by a spectroscopic characterization of the aged material and the detection of Si and Al release into solution. The degraded TiO<sub>2</sub> compounds are able to enter the food chain, where they may exhibit adverse effects.

**Semi-empirical Correlation to Predict the Collision Efficiency of Natural Organic Matter (NOM) - and Polymer-coated Nanoparticles in Porous Media**

Tanapon Phenrat<sup>1</sup>, Charlotte M. Cisneros<sup>1</sup>, Daniel P. Schoenfelder<sup>1</sup>, Robert D. Tilton<sup>2,3</sup>,  
Gregory V. Lowry<sup>1,2\*</sup>

Center for Environmental Implications of Nanotechnology (CEINT) and <sup>1</sup> Department of Civil & Environmental Engineering, <sup>2</sup> Department of Chemical Engineering, <sup>3</sup> Department of Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA 15213-3890, USA.

**Abstract**

Assessing the potential risks of natural or engineered nanoparticles to the environment and human health requires the ability to predict their mobility in porous media such as groundwater aquifers or sand filters used in water treatment. Semi-empirical correlations to predict the collision efficiency of electrostatically stabilized nanoparticles are available; however, they are not likely to be applicable to nanoparticles coated with natural organic matter (NOM) or engineered with polymeric surface coatings because the existing correlations do not account for the electrosteric repulsions afforded by coatings that can inhibit attachment to surfaces. In this study regression analysis of published data on the collision efficiency of NOM-coated latex and hematite particles, and for new data on poly(styrene sulfonate)- and polyaspartate-coated hematite nanoparticles is used to develop a semi-empirical correlation to predict the collision

efficiency of NOM- and polymer-coated nanomaterials that includes the adsorbed layer-electrokinetic parameter ( $N_{LEK}$ ) representing electrosteric repulsions afforded by adsorbed NOM or polyelectrolyte. Despite the large variation in particle type, coating type, and solution conditions, a single semi-empirical correlation with three dimensionless parameters can predict their measured collision efficiency. This study emphasizes the importance of including the adsorbed NOM and polymer layer properties for predicting the collision efficiency of NOM- and polymer-coated natural and manufactured nanomaterials in porous media and offers a functional form of a semi-empirical parameter for such predictions. This modified semi-empirical correlation allows the preliminary estimation of the migration of nanoparticles in porous media once the adsorbed layer properties of surface coatings on the surface of nanoparticles is known.

## **Thermal and Oxidative Stability of Ligand-Modified Nanoparticles: Implications for Environmental Safety of Nanoparticles**

Mangham, Andrew; Wiecinski, Paige; Yang, Sarah; Louis, Kacie; Peterson, Richard E.; Heideman, Warren; Hamers, Robert; Pedersen, Joel; **Hamers, Robert**

The routes of exposure and toxicity of nanoparticles in the environment are expected to be strongly affected by the nature of surface chemical groups exposed on the outside of the particles. We have developed a laboratory-based method that simulates oxidative processes in the environment, and have applied this method to investigate the resulting changes in surface chemistry of “bare” and ligand-functionalized nanoparticles. Using CdSe as a model system, we have compared the behavior of ligands bearing different surface anchor groups including carboxylic acid groups, amines, and phosphonates. Wrapping these ligand-modified nanoparticles with amphiphilic polymers can enhance the particle stability as well as the luminescence efficiency. Surprisingly, the type of surface anchoring group has a strong effect on the stability of the polymer-wrapped nanoparticles. Using infrared and x-ray spectroscopies, combined with thermogravimetric analysis, we related the differences in stability of the nanoparticles to the chemical and physical structure of the ligands. As time permits, the influence of the surface chemistry on the nanoparticle toxicity using a zebrafish model will be presented and discussed.

## Structure affecting reactivity: the fullerene example.

*Ernest M. Hotze and Mark R. Wiesner Duke University*

*Jerome Labille and Jean-Yves Bottero CEREGE, Aix-en-Provence, France*

The chemical behavior of an individual nanoparticle rarely, if ever, carries over faultlessly into the aqueous environment. Aggregation affects the properties of suspended nanomaterials. Therefore, modeling these effects becomes essential to understanding any reactive nanomaterial species released or utilized in an aqueous setting. In this work, we develop a theoretical framework for describing the impact of aggregate structure on the net nanoparticle reactivity in suspension. This theory is illustrated for the case of fullerene nanoparticle aggregates where reactivity takes the form of photosensitization of the  $C_{60}$  molecule. Fractal dimensions are applied to aggregates within the model and utilized to estimate local nanomaterial concentrations and kinetics (Figure 1).

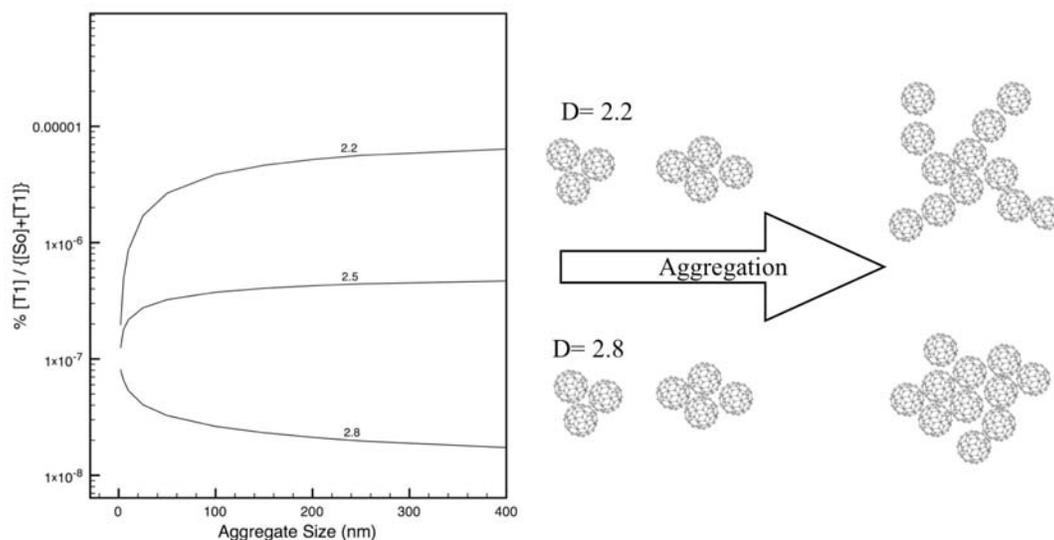


Figure 1: Ratio of  $C_{60}$  cages in the active triplet state  $[T_1]$  over an aggregate size range of 1-400 nm at three fractal dimensions (2.2, 2.5, and 2.8).

The theory resolves the paradox between the high quantum yields observed for reactive oxygen species (ROS) production by  $C_{60}$  compared with hydroxylated  $C_{60}$  (fullerol) observed in organic solutions<sup>1, 2</sup> as opposed to aqueous aggregates of fullerol in suspension which produce relatively large amounts of ROS when compared with aggregates of the undifferentiated  $C_{60}$  that produce no ROS<sup>3</sup>.

## References

1. Arbogast, J. W.; Darmany, A. P.; Foote, C. S.; Rubin, Y.; Diederich, F. N.; Alvarez, M. M.; Anz, S. J.; Whetten, R. L., Photophysical properties of  $C_{60}$ . *Journal of Physical Chemistry* **1991**, 95, (1), 11-12.
2. Palit, D. K.; Mohan, H.; Mittal, J. P., Photophysical properties of  $C_{60}H_{18}$  and  $C_{60}H_{36}$ : A laser flash photolysis and pulse radiolysis study. *Journal of Physical Chemistry A* **1998**, 102, (24), 4456-4461.
3. Hotze, E. M.; Labille, J.; Alvarez, P.; Wiesner, M. R., Mechanisms of photochemistry and reactive oxygen production by fullerene suspensions in water. *Environmental Science & Technology* **2008**, 42, (11), 4175-4180.

# CeO<sub>2</sub> and ZnO nanoparticles undergo differential biological transformations in soybean plants

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Nanoecotoxicology studies the interaction of engineered nanoparticles (NPs) with living organisms (1). Reports have described the toxicity of various nanomaterials on micro and macroorganisms (2, 3), but this information is still scarce. As for plants, the toxicity of some NPs on model plants and crop species (4) has been reported. However, to the authors' knowledge, there is no information about the plant biotransformation of metal NPs. In this study, for the first time, we show evidence pertaining to the biotransformation of ZnO and CeO<sub>2</sub> NPs by soybean seedlings. Although the NPs did not affect germination, they produced a differential effect on plant growth and element uptake. The root length was reduced by 40% in seedlings treated with 4000 mg L<sup>-1</sup> ZnO NPs, but increased by 75% in seedlings treated with 4000 mg L<sup>-1</sup> CeO<sub>2</sub> NPs. At 4000 mg L<sup>-1</sup> treatment, seedlings treated with CeO NPs had 462 mg Ce kg<sup>-1</sup> DW, which was about 3-fold the amount of Zn found in seedlings treated with ZnO NPs (153 mg kg<sup>-1</sup> DW). The synchrotron X-ray absorption spectroscopy showed clear evidence of the presence of CeO<sub>2</sub> NPs in roots, whereas the ZnO NPs were clearly not present within the roots. Our results will assist in elucidating the possible interactions of some nanomaterials with living plants and further explore the potential ability of some plant species to generate defense mechanisms for metal NP detoxification.

## References

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- (2) Reddy, K.M., Feris, K., Bell, J., Wingett, D. G., Hanley, C., Punnoose, A. Selective toxicity of zinc oxide nanoparticles to prokaryotic and eukaryotic systems. *Applied Physics Letters* **2007**, 90(21): 213902/1-213902/3.
- (3) Seeger, E.V., Baun, A., Kastner, M., Trapp, S. Insignificant acute toxicity of TiO<sub>2</sub> nanoparticles to willow trees. *Journal of Soils and Sediments* **2009**, 9:46-53
- (4) Lin, D., Xing, B. Root uptake and phytotoxicity of ZnO nanoparticles. *Environmental Science and Technology* **2008**, 42:5580-5585.

# Effects of Surface Coating and Solution Ionic Composition on Aggregation of Au Nanoparticles in Freshwater Systems

M. Hull<sup>1</sup>, J. Jones<sup>1</sup>, and P. Vikesland<sup>1</sup>

<sup>1</sup> Virginia Tech, Department of Civil and Environmental Engineering, Blacksburg, Virginia, USA.

## Introduction

An improved understanding of how different surface coatings affect the stability and bioavailability of nanoparticles in aquatic systems is required. Gold nanoparticles (AuNP) offer an ideal model system for investigating these phenomena due to: i) the availability of multiple characterization strategies for AuNP, ii) gold background levels in laboratory and environmental systems are inherently low, and iii) a broad spectrum of well-established synthesis and surface functionalization strategies are available for AuNP. The current study examines the stability of AuNP functionalized with citrate or bovine serum albumin (BSA) in a synthetic freshwater diluent used routinely in US EPA standard aquatic bioassay protocols. Our results have implications on assessing the bioavailability of nanoparticles in aquatic ecosystems.

## Experimental

*Preparation of citrate-AuNP and BSA-AuNP.* Gold nanoparticles (AuNP) of 13 to 20 nm nominal diameter were prepared by the citrate reduction of chloroauric acid (1). As-synthesized AuNPs were capped with a citrate coating (citrate-AuNP). Aliquots of AuNP-Citrate were incubated with BSA at 0.1 mg BSA/mL (BSA-AuNP).

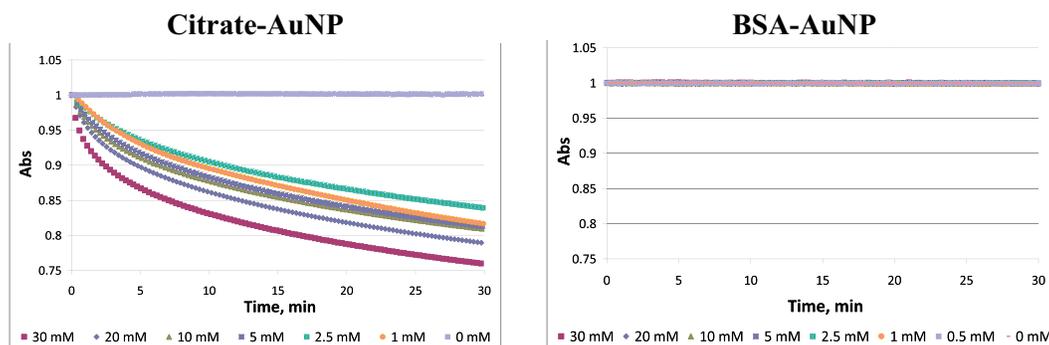
*Characterization.* Following synthesis, UV-VIS-NIR absorbance spectra were obtained for AuNP using a Varian Cary 5000 spectrophotometer. The location and width of the intrinsic surface plasmon peak is indicative of particle size and sample heterogeneity.

*Freshwater Stability and Kinetic Experiments.* The stability of citrate-AuNP and BSA-AuNP was observed in moderately hard, synthetic (MHS) freshwater (2). The kinetic stability of AuNP was determined by tracking the absorbance maxima in solutions containing experimentally varied concentrations (1 to 30 mM) of the individual monovalent and divalent salts from which the MHS was prepared.

## Results

Synthesized batches of citrate-AuNP had absorbance maxima between 520 to 525 nm. The addition of 0.1 mg BSA/mL red-shifted the absorbance maximum to 531 nm. Upon addition of citrate-AuNP (0.6 mM as Au) to MHS the absorbance at 525 nm decreased by more than 20% and was accompanied by an increase in solution absorption at 718 nm. This red-shift in the absorbance maximum is the result of the dipole-dipole interaction among Au aggregates, and is indicative of AuNP coagulation. In contrast, the absorbance maximum of BSA-AuNP remained constant at 531 nm when mixed with MHS, indicating that BSA inhibited coagulation of AuNP in MHS.

Kinetic experiments performed with the individual salts used to prepare the MHS solution indicated that aggregation of citrate-AuNP was rapid and continuous in solutions comprised of divalent cations (e.g.,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ), whereas BSA-AuNP remained well-dispersed in salt concentrations up to 30 mM—the maximum concentration studied (Figure 1). The rate and extent of aggregation of citrate-AuNP in monovalent salt solutions ( $\text{NaHCO}_3$ ,  $\text{KCl}$ ) was reduced relative to the divalent salts ( $\text{CaSO}_4$ ,  $\text{MgSO}_4$ ).



**Figure 1.** Absorbance at 522 nm for AuNP in  $\text{MgSO}_4$  (0 to 30 mM).

## Conclusions

Our findings indicate that citrate-AuNP aggregates rapidly upon addition to a synthetic freshwater diluent specified by US EPA aquatic bioassay protocols. Kinetic experiments with the individual salts comprising the synthetic freshwater suggest that, as predicted by the Schulze-Hardy rule, aggregation of the negatively charged AuNPs is governed primarily by the concentration of divalent cations—in this case,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ . The addition of 0.1 mg BSA/mL, however, dramatically improves the stability of AuNP in the synthetic diluent, with no appreciable aggregation occurring at salt concentrations up to 30 mM. Brewer et al (3) suggested that in the presence of citrate, BSA binds to AuNP by an electrostatic mechanism involving salt-bridges between the citrate present on the gold surface and the 60 surface lysine groups present on BSA. Our results indicate that protein surface coatings can stabilize AuNP in freshwater systems, even at elevated ionic strengths. These findings, which are consistent with well-established principles of colloid and surface chemistry, can be applied to inform nanoparticle dosing strategies in freshwater aquatic bioassays. Ongoing studies will attempt to determine the extent to which nanoparticle stability influences bioavailability and uptake by aquatic biota.

## References

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- (2) EPA. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, Fifth Edition. US EPA Document 821/R-02-012. **2002** US EPA, Washington, DC.
- (3) Brewer, S.H., et al., Probing BSA binding to citrate-coated gold nanoparticles and surfaces. *Langmuir*. **2005** 21:9303-9307.

# Transport of TiO<sub>2</sub> Nanoparticles: Role of Solution Chemistry and Particle Concentration

Yongsuk Hong and Sharon Walker

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## Abstract

The transport of TiO<sub>2</sub> nanoparticles in a quartz-packed column was conducted as a function of pH (5 and 7), ionic strength (1 and 10 mM KCl), and particle concentration (100 and 200 mg/L) at two different flow velocities (2 and 10 mL/min). Results showed that TiO<sub>2</sub> nanoparticles were eluted to a greater extent at lower pH and ionic strength, as well as at higher particle concentration and flow velocity. Specifically, when column tests were conducted with 200 mg/L of TiO<sub>2</sub>, the elution was the greatest in 1 mM KCl at pH 5 and higher flow velocity (i.e., 10 mL/min) with 34.1% of the total particles eluted during the column test. With a low flow velocity (i.e., 2 mL/min) under the same solution chemistry conditions, a total of 13.2% of particles were collected in the effluent. At pH 5, adjusting particle concentration and ionic strength impacted the amount retained in the column. However, at pH 7 results adjustment in the experimental conditions resulted in negligible changes in or levels of elution. To provide further insight into the mechanisms involved in TiO<sub>2</sub> transport, extensive characterization of the nanoparticles (i.e., size, electrophoretic mobility, stability) under this range of solution chemistry and concentration was conducted. Results suggest a combination of rapid aggregation, blocking, and subsequent straining as mechanisms involved in TiO<sub>2</sub> retention in the packed bed column. These results and the implications for nanoparticle fate will be discussed.

## **Oxidation of carbonaceous nanoparticles in the atmosphere: effects of ozonation on C<sub>60</sub> aqueous solubility**

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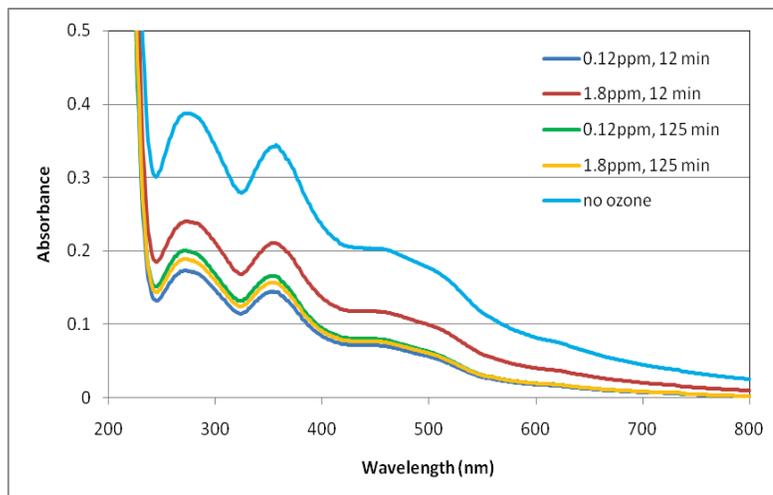
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The potential risks to ecological and human health posed by nanomaterials are a source of concern among researchers and policymakers, as manufactured nanoparticles are likely to enter groundwater, surface water, soil, and air [1, 2]. The C<sub>60</sub> fullerene is used here as a model for carbonaceous nanomaterials because of its relative simplicity, evidence of toxicity, and richer history in the literature. The toxicity of aqueous C<sub>60</sub> fullerene colloids, as well as derivatized fullerenes, has been demonstrated using soil bacteria, fish, and human cell lines [2]. Ionic strength and composition, natural organic matter, and pH have interwoven effects on C<sub>60</sub> colloid size, stability, and morphology, as well as the affinity with which the colloids deposit in media such as sand [2]. Fullerenes also interact with environmental contaminants to varying degrees; uptake is dependent upon the nature of the contaminant as well as the presence of humic acid [3]. Much less, however, is known about fullerenes' fate in the atmosphere.

Airborne carbonaceous nanomaterials are likely to be found in production facilities [4] and in ambient air [5, 6] and may exhibit toxic effects to alveolar cells [5]. When aerosolized, nanomaterials are unlikely to remain as separate primary particles but will instead be present as larger aggregates. Furthermore, airborne nanoparticles may be exposed to oxidants in the atmosphere, such as the hydroxyl radical, ozone, or the nitrate radical, that could transform them chemically. Such reactions could change their toxicity, colloidal formation, deposition, and interactions with other contaminants. In laboratory studies, exposure of C<sub>60</sub> in an aqueous solution to ozone results in more soluble products due to the development of hydroxyl functionalities on the fullerene cage [7].

Considering the potential influence of atmospheric oxidation on the environmental behavior of fullerenes, we aim to investigate the effects of heterogeneous reactions between ozone and powdered C<sub>60</sub> fullerenes on their aqueous solubility. Fullerenes (99.5% pure) were exposed to low and high levels of ozone, in both batch and continuous flow experiments. Following the exposure, fullerenes were stirred in nanopure water for two weeks and filtered. We measured the UV-VIS absorbance of the filtrates. Preliminary results indicate that oxidation does impact solubility, as absorbance is lower for fullerenes exposed to ozone (Figure 1).



**Figure 1:** UV-VIS absorbance of C<sub>60</sub> fullerenes exposed to ozone.

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Session 2: Toxicity/Ecotoxicity

Co-Chairs: Richard Di Giulio and Patricia Holden

Wednesday, September 9, 2009

10:40 a.m. – 4:40 p.m.

Forum 144/146

# FROM ECOTOXICOLOGY TO NANOECOTOXICOLOGY

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For hazard assessment of nanoparticles (NPs) quantitative nanoecotoxicological data are required. The currently existing literature data on toxicity (L(E)C<sub>50</sub> values) of synthetic NPs in environmentally relevant species was evaluated in order to: i) identify tentatively most harmful NPs and most sensitive organism groups and ii) to provide relevant ecotoxicological information for further risk assessment. The focus was set on selected synthetic NPs (nano TiO<sub>2</sub>, nano ZnO, nano CuO, nano-Ag, SWCNTs, MWCNs and C<sub>60</sub>-fullerenes) and organism groups representing main food-chain levels (bacteria, algae, crustaceans, ciliates, fish, yeasts and nematodes).

Altogether 77 effect values was found, mostly for nano TiO<sub>2</sub> (31%), C<sub>60</sub> (18%), nano ZnO (17%), nano-Ag (13%), SWCNTs and nano CuO (both 9%). Only 3% of the available quantitative ecotoxicological information concerned MWCNTs. Organism-wise, 33% of the data concerned crustaceans, 27% bacteria, 14% algae and 13% fish. For all organism groups studied, solubility of CuO- and ZnO- NPs was a key factor in their aquatic toxicity.

On the basis of the 34 median L(E)C<sub>50</sub> values derived from 77 individual values, NPs were ranked according to their lowest median L(E)C<sub>50</sub> value for the above described organism groups: the most harmful were nano ZnO and nano Ag that were classified „extremely toxic“, (L(E)C<sub>50</sub><0.1 mg/l), followed by C<sub>60</sub> fullerenes and nano CuO that were classified „very toxic“, (L(E)C<sub>50</sub>=0.1-1 mg/l). SWCNTs and MWCNTs were classified “toxic” (L(E)C<sub>50</sub>=1-10 mg/l). Nano TiO<sub>2</sub> was classified as „harmful“, (L(E)C<sub>50</sub>=10-100 mg/l). Throughout, algae and crustaceans (daphnids) were most sensitive and thus probably most vulnerable organism groups in aquatic exposure to NPs. Very low L(E)C<sub>50</sub> values should deserve thorough attention of environmental risk assessors for evaluation of the potential adverse effects of synthetic NPs on ecosystems. As the quantitative nanoecotoxicological data are still rare, further studies are needed.

# Using Predictive Toxicological Paradigm to Study Nanotoxicity and Design Safe Nanomaterial

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With the rapid implementation of nanotechnology and the accompanying rate by which commercially available nanomaterials are being introduced, we need a blueprint for safe design of nanomaterials. We advocate a predictive toxicological paradigm for the assessment of nanomaterial hazard and safe design. The predictive toxicological approach is defined as establishing and using mechanisms and pathways of injury at a cellular and molecular level to prioritize screening for adverse biological effects and health outcomes *in vivo*. Specifically as it relates to nanomaterials, a predictive approach has to consider the physicochemical properties of the materials that could lead to molecular or cellular injury and also has to be valid for predicting the possibility of adverse health outcomes in humans and impact organisms. The hierarchical oxidative stress paradigm (HOP) is an example of an injury mechanism that can be used to explore the potential that some types of nanomaterials may generate oxidant injury at intact animal level. As an example, among three metal oxides (ZnO, CeO<sub>2</sub> and TiO<sub>2</sub>), ZnO was clearly the most toxic in HOP screening in bronchial epithelial and macrophages cell lines. This toxicity is premised on particle dissolution and shedding of toxic Zn<sup>2+</sup> that is responsible for cellular ROS production and pro-inflammatory responses. Another example is cationic nanoparticles, including amino-polystyrene and polyethylenimine (PEI)-coated mesoporous silica nanoparticles (MSNP). These particles induce cationic toxicity that is premised on high cellular uptake leading to lysosomal and mitochondrial injury, including cellular ROS production. In addition to serving as a useful test platform for the assessment of nanomaterial hazard, the HOP may be also be of considerable importance for the safe design of nanomaterials. Thus, changing the dilution chemistry of ZnO and cationic density of PEI-coated MSNP can be shown to reduce their toxicity as reflected by HOP screening. This constitutes an ideal nano-EHS paradigm that utilizes quantitative structure-activity relationships for the safe design of nanomaterials that have biological and commercial applications. Examples are decreasing ZnO nanoparticle dissolution by iron doping and attachment of shorter length PEI polymers to the MSNP surface. ZnO nanoparticles are used in sunscreens while PEI coating is useful for the delivery of siRNA and plasmid DNA.

## Developmental toxicity of oxidatively degraded quantum dots

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Considerable uncertainty exists about the extent to which engineered nanomaterials may pose an environmental hazard. We examined the toxicity to zebrafish embryos of prototypical engineered nanomaterials before and after simulated environmental “weathering”. We employed CdSe<sub>core</sub>/ZnS<sub>shell</sub> quantum dots (QDs) functionalized with poly(ethylene) glycol (PEG) of two chain lengths. To examine the environmental weathering of QDs under oxidative conditions, nanoparticles were exposed *in vitro* to a methoxyhydroquinone (MHQ)-driven Fenton’s reaction simulating the extracellular chemistry of lignolytic fungi. Zebrafish embryos were exposed to aqueous suspensions of intact and weathered QDs and assessed daily, for 5 days, for sublethal effects and mortality. Exposure to oxidative conditions severely degraded QDs resulting in the release of Cd<sup>2+</sup> and formation of amorphous Se-containing nanoparticles. Weathered QDs showed higher lethality (LC<sub>50</sub> = 14 μM Cd equivalents) than intact PEG<sub>5000</sub>-QDs (LC<sub>50</sub> = 42 μM Cd equivalents), and both were more toxic than an equivalent amount of cadmium (LC<sub>50</sub> = 409 μM Cd<sup>2+</sup>). Many endpoints of toxicity resembled those induced by cadmium; however, other endpoints of toxicity were also observed. Endpoints were similar for weathered QDs and intact PEG<sub>5000</sub>-QDs. We have eliminated the contributions of Zn<sup>2+</sup> and (oxidized) PEG ligand to the increased lethality of weathered QDs. Increased lethality of weathered QDs may be due to the selenium nanoparticles formed during exposure to the MHQ-Fenton’s reaction.

# **Silver nanoparticle behavior and fish embryotoxicity across a salinity gradient**

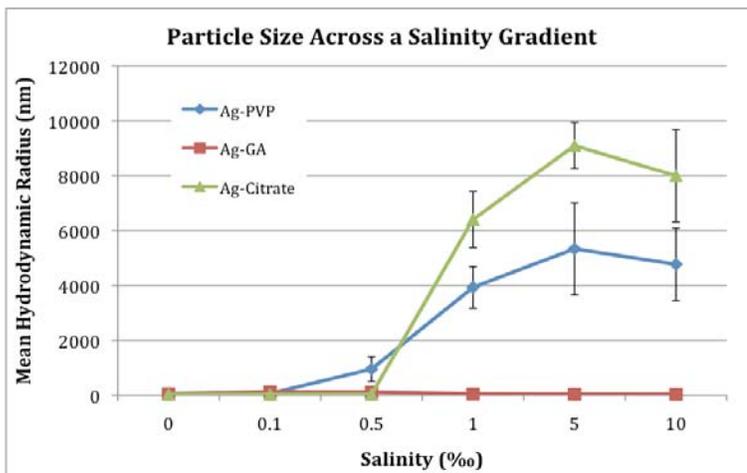
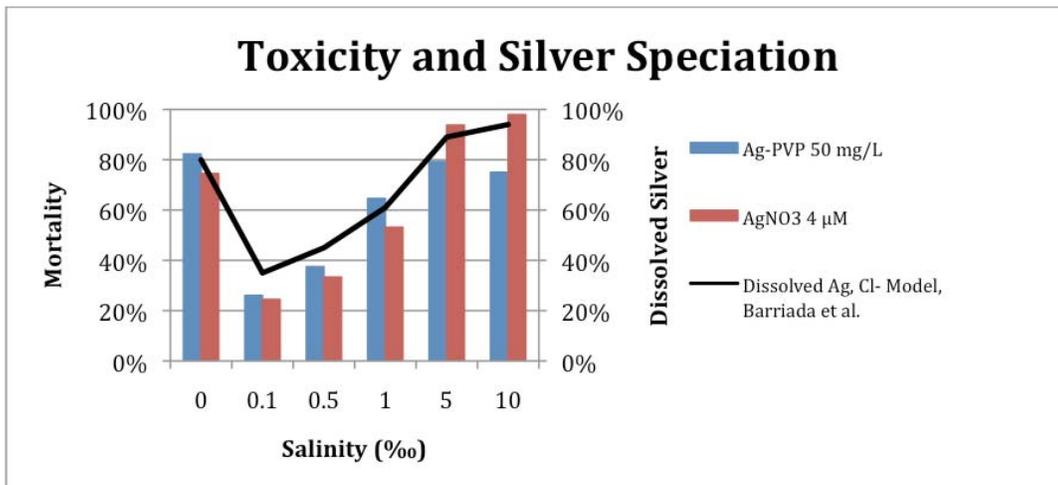
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As silver nanoparticle (Ag-np) production and use increases, a thorough understanding of their potential risk is critical. Although, fish embryotoxicity testing has become a standard method for testing chemical or particle toxicity, many standard fish models tolerate only a small range of environmental variables, including salinity. Atlantic killifish (*Fundulus heteroclitus*) embryos can tolerate extreme variations in salinity, making them an ideal model for testing the influence of salinity on Ag-np toxicity. Silver speciation can be predicted across a salinity gradient. By choosing a salinity that precludes the presence of significant amounts of Ag<sup>+</sup> ions or other dissolved Ag species, any observed toxicity would be attributed to the nanoparticles. Alternatively, when dose response curves track dissolved Ag, one would conclude that non-nanoparticle specific effects are driving toxicity. Previous research with adult fish has shown reduced Ag toxicity with increasing salinity. We hypothesized that Ag-np toxicity would be negatively correlated with salinity, based on both an expected increase in particle aggregation and the loss of Ag<sup>+</sup> ions (through creation of AgCl<sub>n</sub> species) for any Ag leaking from the Ag-np.

Silver nanoparticle aggregation was tested using dynamic light scattering. As expected, Ag-np coated with PVP or citrate aggregated rapidly with increasing salinity. The critical concentrations of coagulation were ~0.1‰ and ~0.5‰ salinity, respectively. However, gum arabic-coated particles did not significantly aggregate with increasing salinity through 10‰. Contrary to our hypothesis, embryotoxicity followed a complex “v” shaped dose response, with toxicity initially decreasing between pure H<sub>2</sub>O and ~0.5‰ salinity. As salinity increases above 0.5‰, toxicity increased and reached a plateau around 5‰ and remained unchanged as salinity increased to 37‰, the maximum tested.

This embryotoxicity is best explained by total dissolved Ag released from the Ag-np. Our data suggest that for PVP-coated particles, dissolution of the Ag-np, and not a Ag-np specific nano-effect, is driving the toxicity observed in our system. Data for the other coatings reveal more complex toxicity, with a combination of dissolved silver and nanoparticle-specific effects. These results suggest that marine and estuarine systems may be particularly susceptible to the embryotoxic effects of Ag-np released into aquatic systems.



# Implications of silver nanoparticles for eleutheroembryos and larval medaka (*Oryzias latipes*)

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The remarkable clarity of the chorionic membranes in the small aquarium model fish, medaka (*Oryzias latipes*) has captured the attention of developmental biologists and environmental toxicologists. Studies on normal and abnormal development, mutagenesis, carcinogenesis, targeted toxicity responses and modes of action of aquatic contaminants illustrate the utility of this model. Despite their transparency, the chorionic membranes comprise an ill-defined, transient and partially protective barrier to entry of nanomaterials in water (e.g. carbon nanotubes) (1). Because the embryonic period only lasts approximately 10 days (at water temperature of 26°C), any protection against nanomaterials in water due to chorionic membranes is brief and the eleutheroembryo (free swimming larval form), devoid of such membranes, must cope with nanomaterials while undergoing further development. Medaka larvae, like larvae of other teleosts, are not small adults. Rather, they must undergo migrational movement of internal organs to adult positions and accommodate growth and elongation while adapting to exogenous food. In addition, larval developmental stages include formation of scales, differentiation of gills, migration of ion regulatory cells, differentiation of gas bladder, redistribution of lipid stores, and remodeling of the homocercal fin to the heterocercal form of the adult. Interestingly, these physiologically important larval stages were given little or no attention in current reviews regarding environmental implications of nanomaterials (e.g. 2). Furthermore, atlases of development have generally focused on earlier stages and not development and differentiation in larvae. Nevertheless experimentally medaka larvae were found to be more sensitive to chemicals than the embryos (3), indicating the need to address this important knowledge gap.

Using the latest Iwamatsu atlas (4) and existing/emerging protocols for larval care and maintenance, work in this laboratory has shifted to a consideration of the eleutheroembryo and larval life stages as a sensitive platform from which to assess environmental implications of nanotechnology with the example of silver nanoparticles (AgNPs). AgNPs have gained much commercial attention and are already in use in numerous consumer products including textiles, personal care products, food storage containers, laundry additives, home appliances, paints, and even food supplements (5). Toxic mechanism of Ag to fish is mainly through inhibition of Na<sup>+</sup>, K<sup>+</sup>-ATPase in the basolateral membrane in the gills by Ag<sup>+</sup> ions, disrupting ion regulation and homeostasis of fish (6). On the other hand, early medaka larvae have poorly differentiated gills, skin without scales but containing ion-regulatory cells, therefore the target site, or even the

mode of action of Ag toxicity may well be life stage dependent. Moreover, this is complicated by recent evidence suggesting that AgNPs exert toxicity through both dissolved Ag ions and the NP itself (6).

Effect of AgNPs on medaka larvae will be presented. By use of the orange red and the see-through mutant (STII) medaka, we shall present findings in surficial and deep tissues within exposed individuals.

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# Effects of Metal Oxide Nanomaterials on Sea Urchin Embryo Development

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Little is known regarding the effects of nanomaterials (NMs) on the development of marine organisms. We have focused on the impacts of metal oxide NMs on the development of embryos from the white sea urchin (*Lytechinus pictus*). Three metal oxide NMs (ZnO, CeO<sub>2</sub>, and TiO<sub>2</sub>) were prepared as stock solutions in filtered sea water with or without 10 ppm alginate, and then diluted into 12-well culture plates for embryo exposure. Newly fertilized sea urchin embryos were exposed to NMs for approximately 96 hours, until the pluteus stage, and then assessed for normal development. ZnO was toxic to developing sea urchins as low as 1-10 ppb, with greater than 50% of embryos showing abnormalities at 100ppb. Using alginate as a source of dissolved organic carbon (DOC), we found that DOC increased the toxicity of ZnO. Two other metal oxide NMs, TiO<sub>2</sub> and CeO<sub>2</sub>, had no effect on development up to 50 ppm, and alginate did not increase their potency. The effects of ZnO NM on development did not appear to be stage-specific but rather, the effects were a result of exposure duration. Finally, using ZnO NMs that were covalently conjugated with FITC, we found that FITC-ZnO NM was incorporated into cells of the embryos. Using scanning laser confocal microscopy, we found ZnO-FITC fluorescence throughout the embryo, with a few select cells showing a much greater accumulation of FITC-ZnO. FITC-ZnO was excluded from the nuclei of cells.

# Silver nanoparticles inhibit growth in *Caenorhabditis elegans*

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We are studying the ability of silver nanoparticles of different size and coatings (PVP and citrate) to inhibit the growth of *Caenorhabditis elegans*, a species of nematode commonly used in laboratory studies that offers many advantages for toxicological studies (1). All tested silver nanoparticles resulted in growth inhibition detectable beginning between 5 and 25 mg/L, when larval growth was assayed in K<sup>+</sup> liquid medium (2). Due to the elevated ionic strength and the neutral pH, all three types of silver nanoparticles are physico-chemically destabilized in the K<sup>+</sup> medium. Significant aggregation occurs as a function of the concentration and the incubation time. Moreover, the equilibrium of the silver nanoparticles within the solution results in the release of silver ions detectable at doses of particulate silver that caused toxicity.

We also observed that these levels of silver nanoparticles were toxic to the *Escherichia coli* that *C. elegans* eats. Heat-killed *E. coli* could not be used because *C. elegans* grows poorly on heat-killed *E. coli*. We are currently carrying out studies designed to identify an alternate mode of killing *E. coli* that will support optimal growth for *C. elegans*.

In addition to using a wild-type strain of *C. elegans* (N2), we are testing nanoparticle toxicity using 5 additional strains that carry mutations in genes coding for proteins involved in key defensive pathways: DNA repair-deficient strains (*xpa-1*, *nth-1*), a metal-sensitive strain (*mtl-2*), and two oxidant-sensitive strains (*sod-2*, *mev-1*). Preliminary data suggests that the *mtl-2* strain is the only strain that is more sensitive than wildtype to silver nanoparticle exposure.

We will present our test protocol schematically, and present the results of the experiments discussed above as well as ongoing work.

## Acknowledgments

We gratefully acknowledge the work of Elena Turner in adapting the growth assay to the COPAS Biosorter (“worm sorter”) and in troubleshooting the use of UVC-killed bacteria as a food source.

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# Ecotoxicity of altered TiO<sub>2</sub> nanocomposite on the earthworm, *Eisenia fetida*

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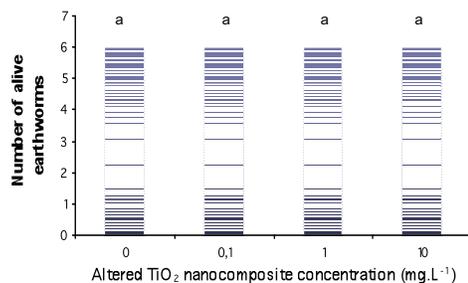
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The development and production of nanomaterials in medical science, cosmetic industry and engineering is in constant increase (1). There is an interest in considering the impact of nanomaterials on environment and human health (2). Surprisingly, ecotoxicological risks associated to the alteration and aging of nanomaterials are poorly documented.

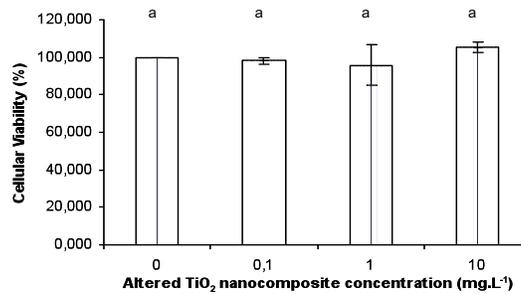
Our aim was to evaluate the potential toxicity of altered TiO<sub>2</sub> nanocomposite initially contained in a manufactured sunscreen cream on the earthworm *Eisenia fetida*, taking account survival and immunotoxicity parameters.

In soils ecotoxicology, earthworms are used as suitable biomonitors (3) because of their low cost, easy handling and the standardization of ecotoxicological tests (4). In order to simulate the alteration of the nanocomposite present in the sunscreen cream, an accelerated aging process was realized by mixing TiO<sub>2</sub> nanocomposite with water during 48h. Then, a stable solution of TiO<sub>2</sub> rich nano-residus was obtained, chemically and physically characterized. Earthworms have been exposed to altered nanocomposite solutions at 4 concentrations (0, 0.1, 1 and 10 mg.L<sup>-1</sup>), during 24h in the dark. For each concentration, six earthworms were introduced in petri dishes containing the solution (5 replicates per concentration). The viability of cœlomocytes has been measured by the MTT assay and phagocytosis has been evaluated by microscopic observations of phagocytosed fluorescent latex beads.

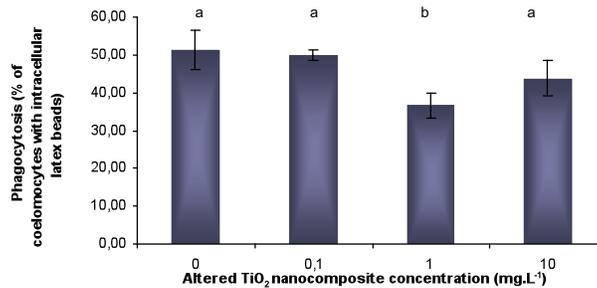
Results showed that the altered TiO<sub>2</sub> nanocomposite had no effect on survival rate (fig.1) and did not induce cytotoxicity on cœlomocytes (fig.2). By contrast, a significant decrease of phagocytosis activity was observed at 1 mg.L<sup>-1</sup> (fig.3).



**Figure 1:** Number of surviving earthworms, *Eisenia fetida*, after 24h of exposure to altered TiO<sub>2</sub> nanocomposite (0, 0.1, 1 and 10 mg.L<sup>-1</sup>). Different letters indicate significant differences between concentrations ( $p < 0.05$ ).



**Figure 2:** Cellular viability of cœlomocytes (*Eisenia fetida*) exposed *in vivo* to altered TiO<sub>2</sub> nanocomposite (0, 0.1, 1 and 10 mg.L<sup>-1</sup>) during 24h. Different letters indicate significant differences between concentrations ( $p < 0.05$ ).



**Figure 3:** Phagocytosis activity of cœlomocytes (*Eisenia fetida*) exposed *in vivo* to altered TiO<sub>2</sub> nanocomposite (0, 0.1, 1 and 10 mg.L<sup>-1</sup>) during 24h. Different letters indicate significant differences between concentrations ( $p < 0.05$ ).

This study has been realised at pH 7.5, which is the isoelectric point of the nanocomposite. The aggregation of nanoparticles could explain the lack of effect observed on earthworms survival and cytotoxicity.

Phagocytosis activity is a sensitive parameter to evaluate sublethal effects of altered TiO<sub>2</sub> nanocomposites.

In conclusion, this short-time exposure may induce sublethal effects on earthworms at low concentrations. Therefore the expression of enzymes involved in the oxidative stress defence will be studied.

### Acknowledgments

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# Investigation of nanoparticles and carbon nanotubes toxicity and transfer in plants

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Nanoparticles are introduced in a growing number of commercial products, and their production would lead to their release in the environment (air and surface waters). Literature concerning their potential effects on human health is rapidly increasing, however little is known on their fate and effects on the ecosystems. Plants represent a potential entry point for nanoparticles in the food chain. Up to now, about 20 articles relate of their phytotoxic effects with contradictory results. For instance, for Zheng *et al.* (1), TiO<sub>2</sub> exposure has a positive impact on germination rate, biomass and photosynthesis of *Spinacia oleracea* but Lin and Xing (2,3), Lee *et al.* (4) or Asli and Neumann (5) find the opposite with various nanoparticles and plants.

This study was performed on four different plant species. Wheat (*Triticum aestivum*) was chosen as model for monocotyledon and rapeseed (*Brassica napus*), Indian mustard (*Brassica juncea*) or *Arabidopsis thaliana* as model for dicotyledon. Two types of nanoparticles were tested at different concentrations: titanium dioxide (TiO<sub>2</sub>) and multi-walled carbon nanotubes (MWCNT). The first step of the project was to find a method to obtain a well dispersed suspension of nanoparticles and to precisely characterize them. The best results were obtained by dispersing MWCNT in water with Arabic gum (0.25%) whereas TiO<sub>2</sub> were only dispersed in ultrapure water.

Then, different parameters were assessed to represent the impact of nanoparticles exposure on various plant physiological parameters: germination rate, roots elongation, biomass (of roots and shoots) and evapotranspiration. First results show that there is no significant effect on germination rate for all species but there is a trend of positive effect of both MWCNT and TiO<sub>2</sub> on wheat, rapeseed and mustard for other criteria. These nanoparticles have a negative impact on *Arabidopsis thaliana*. These results still need to be confirmed.

Future experiments will address nanoparticles accumulation and distribution in plants which will be investigated by ICP-MS (Inductively Coupled Plasma- Mass Spectrometer) and TEM (Transmission Electronic Microscopy) to see if nanoparticles are able to penetrate in the plants and be translocated in different tissues.

Taken together, all these data will provide valuable information on nanoparticles penetration, effects on plant and fate, permitting to draw conclusions on ecotoxicological risk associated to nanoparticles dissemination.

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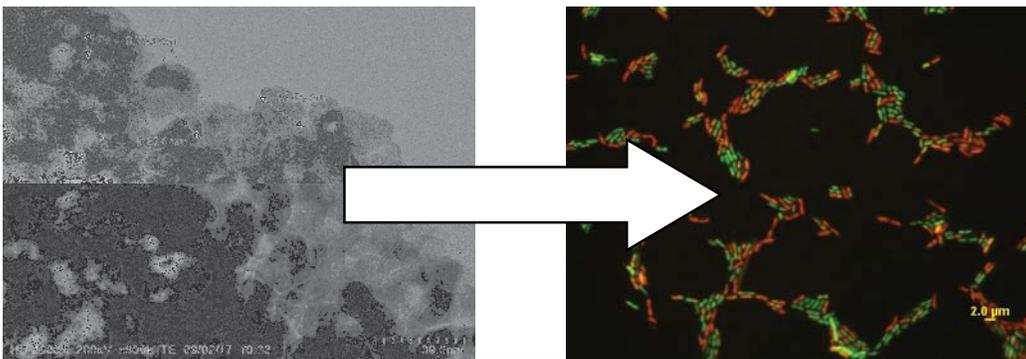
# Impact of metal oxide nanoparticles on microbial cell viability

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The focus of this project is on inorganic nanoparticles, specifically metal oxides (e.g., zinc oxide and titanium dioxide), which have been incorporated into personal care products for UV protection and other consumer products such as photocatalytic coatings, electronics, water decontamination media, and biomedical equipment. Although the eventual disposition of nanoparticles will likely be the environment, minimal research to date has investigated the environmental fate of nano-sized metal oxides. **One significant area of concern is the microbiological fate of metal oxide nanoparticles, specifically their bioavailability and toxicity under environmentally relevant conditions.** Microorganisms perform many critical roles which are required for healthy ecosystem function including nutrient cycling and litter decomposition. In fact, considerable research data indicate that nanoparticles exhibit antibacterial activity toward pure cultures including *Escherichia coli*, *Pseudomonas aeruginosa*, and *Streptococcus aureus* (1, 2). Further, metal oxide nanoparticles have been incorporated into consumer products for their bactericidal properties (3,4). However, the complex nature of the environment has yet to be incorporated into this research.

In this research, we employed direct and in situ detection strategies for observing nanoparticle behaviour and to determine metal oxide nanoparticle toxicity to bacteria. Specifically, we used transmission electron microscopy and fluorescent microscopy for sample characterization before, during, and following microbiological exposure to metal oxide nanoparticles. Titanium dioxide (TiO<sub>2</sub>) and zinc oxide (ZnO) nanoparticles (NPs) were acquired from Sun Innovations Inc. (Fremont, CA) and characterized using TEM (Hitachi HD2300A) (see figure below). The Ti-NPs were found to be polydispersed and in the size range of 10-20nm. Experiments were conducted with various concentrations of NPs (0, 10, 50, 100, and 500 mg/L) in an effort to determine a toxicity threshold for



(Right) TEM of titanium dioxide NPs used in this study. Picture was taken with Hitachi HD 2300A at 200kV and 800k X magnification . (Left) Microscopic (1000X) image of *e.coli* exposed to 10mg/L titanium dioxide NPs. Viable (green) and non-viable (red) cells were identified using a membrane integrity stain (propidium iodide) and a non-specific stain (picogreen).

pure cultures of *E. coli* (gram negative) and *E. faecium* (gram positive). Fluorescent microscopy was used to determine bacterial quantity and viability following 1 hour of NP exposure in pure cultures (laboratory grade water) and in complex samples (natural organic matter). Propidium Iodide (Sigma Chemical Corp.), a red indicator stain ( $\lambda = 617\text{nm}$  maximum), was used to evaluate the viability of cells based on membrane integrity (5). Samples were stained with PI (10 $\mu\text{g}/\text{mL}$  commercial solution) and counterstained with Picogreen (PG; P-7859; Molecular Probes, Inc., Eugene OR) (4 $\times 10^{-3}$  of stock) for viability assessment. Bacteria that maintained their membrane integrity produced green fluorescence while organisms with permeable and damaged membranes fluoresced red and orange, respectively (see microscope image).

The data suggest that with increased concentration of NPs, the number of viable *E. coli* cells (green only) decreases in comparison with the control samples that were not exposed to NPs. Results of this experiment revealed a measurable impact on *E. coli* viability at 50 mg/L TiO<sub>2</sub> and 100 mg/L ZnO. Notably, the same result was achieved in the presence of natural organic matter (NOM), which was added to increase sample complexity. Suwannee River Humics (Standard II, International Humic Substances Society) was used as a model natural organic matter. The total organic carbon content of the solution was found to be 2 mg/L typical of natural surface waters. On the other hand, no statistically significant ( $\alpha = 0.05$ ) decrease in the number of viable cells was observed with the gram positive bacteria (*E. faecium*) in the presence of either ZnO or TiO<sub>2</sub> NPs. Ongoing studies involve assessing the impact of TiO<sub>2</sub> and ZnO NPs on cell activity and cell function in complex systems such as wastewater processes.

Nanotechnology is a dominant area of research in the fields of science and engineering. A significant amount of resources, allocated by both public and private agencies, have been directed toward advances in the development and manipulation of nanoparticles resulting in a variety of nanotechnologies with considerable potential. However, it is unclear as to whether the benefits of nanotechnology outweigh the risks associated with nanoparticles.

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Session 3: Risk Perception, Risk Assessment & Life Cycle  
Assessment

Co-Chairs: Elizabeth Casman and Hilary Godwin

Wednesday, September 9, 2009

10:40 a.m. – 4:40 p.m.

Auditorium 151/155

# Linking Risk Assessment, Decision Analysis and Life Cycle Assessment for Guiding Nanomaterials Development

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## Abstract

There is rapidly growing interest by regulatory agencies and stakeholders in the potential risks associated with nanomaterials throughout the different stages of products' life cycle (e.g., development, production, use and disposal). Risk assessment methods and tools developed and applied to chemical and biological agents may not be readily adaptable for nanomaterials because of the current uncertainty in identifying the relevant physico-chemical and biological properties that adequately describe the materials. Such uncertainty is further driven by the substantial variations in the properties of the original material because of the variable manufacturing processes employed in nanomaterial production. We propose a decision support system for classifying nanomaterials into different risk categories. The classification system is based on a set of performance metrics that measure both the toxicity and physico-chemical characteristics of the original materials, as well as the expected environmental impacts through the product life cycle. Ability to relate physico-chemical characteristic of the materials to product life cycle risk could be used to guide nanomaterial development.

# **Estimating Nanomaterial Emission Sources from Production Information**

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Though it is a critical step in assessing the environmental risks from nanomaterials, there has been very limited work thus far toward quantitatively estimating their release. Our work presents a model for the physical flows of nanomaterials throughout their lifecycles, and asserts that upper bounds on the potential nanomaterial emissions can be defined based on production volumes.

We have culled information from company websites, interviews, patents, and publications to estimate current production volumes for three classes of materials: TiO<sub>2</sub>, Nano-Ag, and C<sub>60</sub>/CNTs. Here we introduce a model representing the possible flows of these materials from their sources through the specific pathway of wastewater treatment facilities and out into the environment either as sludge or as effluent water. We further introduce a plan for measuring influent and effluent concentrations of a nanomaterial in a wastewater treatment plant in order to scale this difference to our source volume estimates on a per capita basis.

The goal of this work is to set up a model to allow increasingly realistic predictions of nanomaterial concentrations in particular environmental compartments through updated data on production volumes and WWTP influent and effluent concentrations.

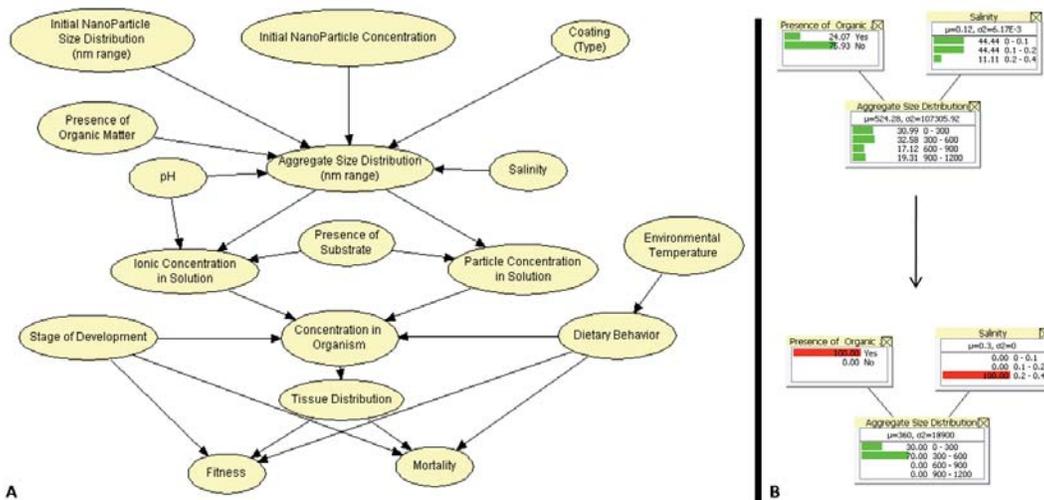


Fig. 1. (a) BayesNet for a biotic response module (a); (b) Data-based probability characterization of the 'Aggregate Size Distribution' node.

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# **Nano-risk and macro-uncertainty: Using probability networks to model the environmental implications of nanotechnology**

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Nanotechnology and its potential to release nanoparticles into various environmental media is an increasing concern among government and public stakeholders. While there are widely known benefits of using nanotechnology, the environmental risks associated with the use of such materials are still uncertain. The characterization of nanoparticles and their subsequent fate, transport, uptake, and exposure through environmental components are continuously being investigated.

Given the amount of uncertainty and the relative infancy of nanoparticle research, there is a need for an adaptable risk assessment framework that can account for this uncertainty and be easily modified as new evidence becomes available. One such framework employs the use of probabilistic networks, commonly known as Bayesian Networks or BayesNets. Bayesian networks are directed acyclic graphs that express the probabilistic relationships between a set of variables. The resulting probability matrix is then used in the propagation of evidence throughout a given Bayesian network structure (1,2).

This work describes the structural development of a modular probabilistic network model that connects information related to particle characteristics, environmental conditions, and bacterial/biotic responses to nanoparticles, using a combination of expert elicitations and experimental data. It involves an iterative process of determining important variables and the connections between them. Various sub-models are introduced, such as the biotic response module appearing in Figure 1a. In addition, we show the diagnostic and predictive capabilities of a BayesNet framework using a simple case study of fullerol aggregate formation in the presence of organic matter and salinity changes (Figure 1b) (3).

A simplified network is proposed that will serve as the guiding model for incorporating the more complex modules related to particle characteristics, environmental conditions, and ecosystem responses. Constructing the model in this way allows for decomposability and facilitates efficient model refinement. In addition, it provides a simplified platform for introducing the modeling results to decision-makers and other stakeholders concerned with the environmental risks of nanomaterials. Given the many complexities in

developing a large-scale model for this situation, further considerations that we address include variable reduction, model generalization, and redundancy.

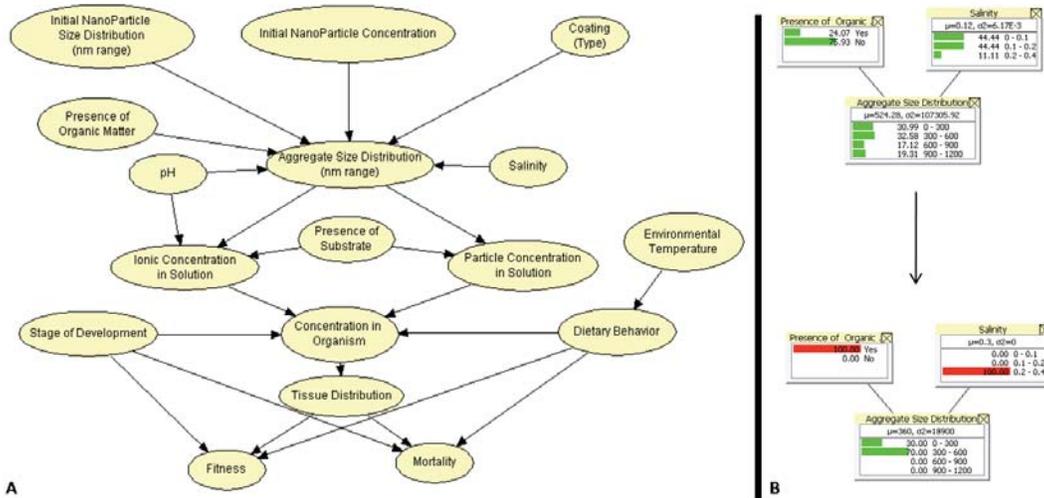


Fig. 1. (a) BayesNet for a biotic response module (a); (b) Data-based probability characterization of the 'Aggregate Size Distribution' node.

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# What nanoparticles merit special considerations from an environmental, health, and safety perspective?

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The regulation of engineered nanoparticles requires a widely agreed definition of these particles. An engineered nanoparticle is usually defined as (i) *any intentionally produced particle that has a characteristic dimension from 1 to 100 nm and that (ii) has properties that are novel, unique, or new compared to the non-nanoscale form of a particle of the same chemical composition*. While part (i) of this fairly broadly accepted definition specifies a size range that is often the focal point for defining nanoparticles, the premise of this communication is that it is the part (ii) that is key in determining which particles merit special consideration from a research, regulatory, and environmental, health and safety perspectives under the banner of nanotechnology.

Indeed, from the viewpoint of assessing possible risks to environment and human health, a key question posed by the introduction of nanoparticles is, do they exhibit properties affecting either exposure or hazard that are fundamentally different from those exhibited by particles of identical composition in their larger, “bulk” format? This remains an open question. While nanoparticles may be more easily taken up by organisms through ingestion, respiration or both, and potentially increases their residence time and exposure in environmental systems, these effects typically result from their small size (extrinsic property) rather than a unique nano-scale property (intrinsic property). New fundamental physics or theories beyond those encompassed by

colloid chemistry are not necessarily needed to describe the interactions of particles in the 1 to 100 nm size range with other materials in the biosphere. However, the novel properties of nanoparticles, their atypical surface structure and reactivity (part (ii) of the definition) may enhance processes such as dissolution, redox reactions, or reactive oxygen species generation. Such novel properties may be accompanied by biological effects that would not be produced by larger particles of the same chemical composition. In these cases, new approaches are needed to systematically define nanoparticles and their properties (*e.g.* structural characterization) as a basis for ensuring the reproducibility of results, identifying underlying mechanisms of toxicity, and predicting environmental behavior.

We focus on inorganic metal and metal oxide nanoparticles for which there is substantial interest in commercial development as well as concerns surrounding their (eco)toxicological impacts. We conclude that there is a critical size, considerably smaller than 100 nm for which these new properties typically appear. This critical size is strongly related to the exponential increase of the number of atoms localized at the surface as the size decrease and delineates a smaller set of nanoparticles (typically diameters less than 20-30 nm). These nanoparticles smaller than 20-30 nm have a size-dependent crystallinity that exhibits properties drastically different from bulk material and fit the two-part definition previously discussed. These observations suggest that nanotoxicological studies might be better focused on a smaller set of nanoparticles that exhibit unique nano-scale properties.

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# **The Impact of Toxicity Testing Costs on Nanomaterial Regulation**

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Information about the toxicity of nanoparticles is important in determining how nanoparticles will be regulated. In the U.S., the burden of collecting this information and conducting risk assessment is placed on regulatory agencies without the budgetary means to carry out this mandate. In this paper, we analyze the impact of testing costs on society's ability to gather information about nanoparticle toxicity and whether such costs can reasonably be borne by an emerging industry. We show for the United States that costs for testing existing nanoparticles ranges from \$249 million for optimistic assumptions about nanoparticle hazards (i.e., they are primarily safe and mainly require simpler screening assays) to \$1.18 billion for a more comprehensive precautionary approach (i.e., all nanomaterials require long-term in vivo testing). At midlevel estimates of total corporate R&D spending, and assuming plausible levels of spending on hazard testing, the time taken to complete testing is likely to be very high (34-53 years) if all existing nanomaterials are to be thoroughly tested. These delays will only increase with time as new nanomaterials are introduced. The delays are considerably less if less-stringent yet risk-averse perspectives are used. Our results support a tiered risk-assessment strategy similar to the EU's REACH legislation for regulating toxic chemicals.

# Real Risk, Perceived Risk, and Nanotechnology: Lessons from Current Research and Past Environmental Controversies

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In his book, *A New Species of Trouble*<sup>1</sup>, Kai Erikson argued that when risks cannot be detected with the five human senses (i.e., they're invisible, un-smellable, etc. to ordinary senses) they will pose a new kind of 'trouble' for citizens and society. Citizens in these cases are forced to depend both on specialized technical equipment and the folks who operate that specialized technical equipment for knowledge and management of the risks. In these cases, people show significantly increased sensitivity to organizational screw-ups, or what Freudenburg calls 'recreancy'<sup>2</sup>. Nanomaterials as nascent risk objects have captured the interest of risk perception researchers precisely because they seem likely to embody a set of factors that have been highly predictive of lowered acceptability and amplified risk perception in past technological cases. In addition to their invisibility, these factors include involuntary exposure, inequitable distribution of risks and benefits, inescapability of exposure, unfamiliarity, man-made origins, violates 'dose makes the poison' assumptions, and appearing to be poorly understood by science<sup>3,4</sup>. And yet there are also many intriguing uncertainties and novel aspects to nanomaterials and what is known thus far about public response to their perceived benefits and risks. IRG 7 research focuses on both emergent perceptions of environmental risk from nanomaterials among different groups of citizens and experts and the comparative historical analysis of past controversies that may shed light on those responses. This presentation will discuss initial analyses of the past controversies, indicating how they and parallel public perception research by the group in the Center for Nanotechnology in Society at UCSB contribute to UC CEIN's understanding of environmental risk perception.

As was the case for the early days of nuclear power (or for that matter with biologically engineered stuff in the U.S. so far, although not in Europe), most people are continuing to

express relatively little concern about nanotechnologies -- but as with nuclear technologies in the later 1970s or with BSE and then with "Frankenfoods" in Europe, initial complacency can easily be turned into a steeply heightened state of concern in quite short order, often through just one or two examples of what Paul Slovic years ago termed "signal" events, namely those that send a signal to the broader public that things might not be as well-controlled as all of us might like. These "non-empirical" technological risks (such as those involving obligatory mediation by machines and expert interpreters) are important for understanding both earlier risks and nano risks, and hence for addressing CEIN risk communication challenges ahead.

Perceptions of benefit also figure significantly in emergent risk perception in ways that are not yet well understood. For example, Gaskell<sup>5</sup> argues that the European amplification of risk around GMOs was in fact driven not by high perception of risk but rather by low perception of benefit. Our CNS group has also found evidence that benefit perception is tempered not by technological risk but by social risks such as perceived injustice in the distribution of benefits.<sup>6</sup> IRG 7's analyses of the key factors in emergent nanotech risk perception thus assess both perception of benefit and perception of risk issues. The presentation will discuss the group's work to date and plans integration of them into nanomaterials environmental risk perception survey research in year 2.

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# Nanotoxicity Assessments with a Whole Zebrafish Model

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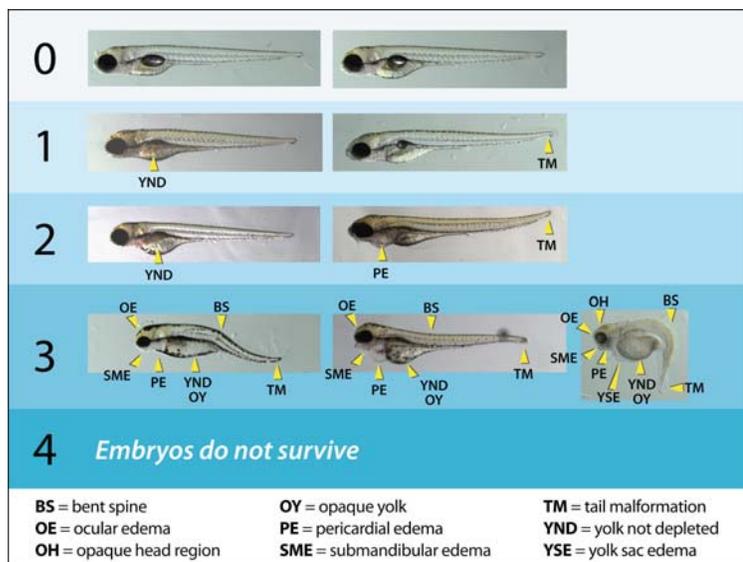
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The continued development of nanoparticles as therapeutics necessitates rapid, correlative, and predictive evaluation methods for toxicity. Current drug pharmacology and toxicity tests use preclinical *in vitro* human cell culture data and *in vivo* mammalian studies extrapolated to human toxicity effects. This established toxicity paradigm limits rapid translational development of nanotherapeutics and other biomaterials used as sub-micron drug carriers with materials sizes approaching the dimensions of proteins. A predictive “methods toolkit” correlating disparate nanomaterials’ physicochemical characteristics (e.g. size, shape, and molecular weight) with toxicity risk assessment is deficient. Development of a medium-throughput set of standard protocols to screen nanomaterials could significantly advance pre-clinical development of nanotherapeutics.

Our research focus is to establish a correlative model between putative *in vitro* and *in vivo* human nanotoxicity mechanisms utilizing model silver nanoparticles (AgNPs) and a novel, zebrafish whole organism model. This study will elucidate the relationships



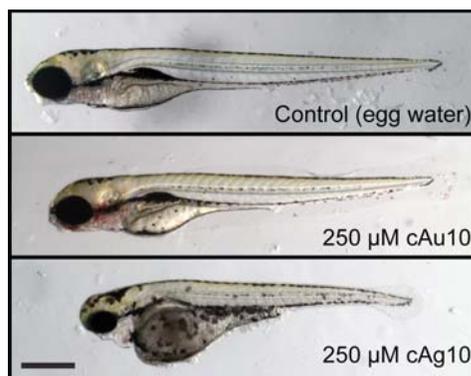
**Figure 1** Representative scoring system for zebrafish toxicity upon exposure to nanoparticles (1).

to identify phenotypic and genotypic abnormalities resulting from exposures to specific nanomaterials by various routes. Experimental findings are validated against nanotoxicity data derived from routine *in vitro* mammalian cell lines. Endpoints of toxicity will be assessed using a novel scoring system (**Figure 1**) for gross developmental

between nanoparticle chemistry and zebrafish physiology and development. Zebrafish (*Danio rerio*) possess a high degree of homology to the human genome and represent an established and validated human surrogate model in developmental biology and molecular genetics. Significantly, both embryonic and adult zebrafish can serve as an ideal model system for whole organism nanotoxicology to

morphological abnormalities; cell-stress markers such as reactive oxygen species production and immunogenicity; biodistribution; and identification of genetic and transduction pathways perturbed by nanoparticle nanotoxicity. Zebrafish embryos are proposed in these studies due to their demonstrated exquisite sensitivity to stressors and chemicals. With their available mutants, genetic manipulations, and microarray assays, zebrafish models have the potential to become the leading organism in biomaterial nanotoxicology.

The diverse physiochemical properties and functions associated with nanoparticles produces an immediate need to assess their potential toxicities. Transparent zebrafish embryos possessing a high degree of human genome homology offer an economically feasible, medium throughput screening platform for non-invasive real-time assessments of toxicity. Using AgNPs and AuNPs in various sizes (3, 10, 50, and 100 nm) and the semi-quantitative scoring system (**Figure 1**), we found that Ag<sub>3/10/50/100</sub> produced almost 100% mortality at the assay termination, while Au<sub>3/10/50/100</sub> produced less than 3% mortality at the same time point (2). Furthermore, while AuNPs induced minimal sublethal toxic effects, AgNP treatments generated a variety of embryonic morphological malformations including stunted growth, opaque and nondepleted yolks, circulatory malformations, jaw defects, and others post-hatching at 5 day incubation (**Figure 2**). Uptake of AgNPs and AuNPs by the embryos and vector control experiments suggested that AgNP toxicity was caused by the nanoparticles themselves or Ag ions formed either during *in vivo* nanoparticle destabilization or residual Ag ions from the synthesis. Although AgNP toxicity was slightly size-dependent at certain concentrations and time points, the most striking result is that parallel AgNP and AuNP sizes induced significantly different toxic profiles, with the former being toxic and the latter being inert in all exposed sizes. Therefore, we concluded that nanoparticle chemistry is as, if not more, important than specific nanoparticles at inducing toxicity *in vivo*. Ultimately such assessments using the zebrafish embryo model should lead to the identification of nanomaterial characteristics that afford minimal or no toxicity and guide more rational designs of materials on the nanoscale.



**Figure 2** Zebrafish images at 5 days per fertilization with continual exposures of 250 mM of 10 nm spherical gold and silver nanoparticles.

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Session 4: Natural Nanomaterials &  
Nanobiogeochemistry

Co-Chairs: Michael Hochella, Jr. and  
Jorge Gardea-Torresdey

Thursday, September 10, 2009

9:00 a.m. – 12:20 p.m.

Room 148/150

## **Microbially mediated transformation of metal and metal oxide nanoparticles**

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### **Abstract**

Engineered nanostructures have a central role in energy conservation strategies and economic growth. One of the most significant impacts of engineered nanostructures is for effecting heterogeneous catalysis as required for fuel transformation, energy storage, polymer production and chemical synthesis. Metal and metal oxide nanoparticles are often used. They possess high surface areas and the ability to selectively mediate chemical transformations. The size and composition of the particle affects performance and may similarly affect nanoparticle fate and transport in the environment. The transformation of such nanoparticle catalysts in the environment is likely to proceed through interactions with bacteria. Several bacterial species are well known to interact with nanoparticles. Nanoparticle production, nanoparticle toxicity, nanoparticle binding and incorporation with bacteria have all been observed. However, basic knowledge that would allow prediction of the probable interaction between an engineered nanoparticle and bacteria is lacking. Our efforts seek to quantify and characterize interactions between engineered metal and metal oxide nanoparticles and selected microbial species. The effect of size and chemical composition of nanoparticles that are currently considered for various applied uses are being studied. Initial efforts are focused on the effects of cerium oxide nanoparticles on the growth, viability and genetic response of *E. coli*. Well-characterized CeO<sub>2</sub> nanoparticles of various sizes have been prepared and presented to bacterial cells in a dose dependent manner. Advanced imaging techniques are used to evaluate the binding and fate of the nanoparticles and the bacterial cell. The results of these studies will provide a basis for understanding how nanoparticle size and composition influence their interactions with microorganisms, and how microorganisms may alter the fate and transformation of engineered nanoparticles in the environment.

# **Nanominerals, mineral nanoparticles, and Earth systems: A partial but useful guide to the behavior of anthropogenic nanomaterials in the environment**

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The explosive growth of nanotechnology has been driven by the rapid development of thousands of different kinds of nanomaterials, several with highly interesting properties having one to enumerable commercial applications. The detailed mechanical, electrical, and chemical characteristics of these intriguing materials are being firmly established. The study of their interactions with living things, as well as determining their general ecological impact in a highly complex Earth environment, is starting to become established, but there is a long way to go (1).

At the same time, naturally occurring nanoparticles are already ubiquitous in the hydrosphere, atmosphere, and soil environments of the Earth as a result of mineral weathering, microbial activity, nucleation/crystallization processes, and anthropogenic activities, etc. (2). Perhaps it is not surprising that there are a number of the same or very similar nanomaterials that are both 1) synthesized and studied for/used in commercial applications, and 2) found naturally in the environment while playing important roles in geo- and biochemical reactions and cycles. These include certain metals (e.g. Au and Ag), oxides (e.g. Fe, Ti, Si, Ce, and Zn oxides), sulfides (e.g. Pb, Fe, Zn sulfides), and carbonaceous materials (e.g. C60). These nanomaterials provide a valuable link between the study of the environmental implications of nanotechnology (1) and nanogeoscience (2) and make it apparent that these two fields are in fact highly complimentary. With approximately 4,500 mineral species presently described, there are probably many more connections between the smallest of minerals and synthetic nanomaterials.

We are already well aware of the influence of minerals in general on all ecosystems on Earth. Minerals exert their influence by constituting the bulk of this rocky planet and having a wide range of composition and structure that is expressed in a remarkable diversity of physical and chemical properties. However, only recently have we been gaining a much better appreciation for another aspect of mineral diversity, that expressed in the nanoscale size range. Here, atomic and electronic structure of nanoparticles may vary with size even without a phase transformation, and surface to volume ratios change dramatically. Such particles are minerals that are as small as approximately one nanometer and as large as several tens of nanometers in at least one dimension. Limiting size in one, two, or three dimensions results in a nanofilm (or nanosheet), a nanorod, or a nanoparticle, respectively. Minerals can be found in all of these shapes. Nanominerals are defined as minerals that only exist in this size range; that is, one will not find their equivalent at sizes larger than this. Well-known examples include certain clays and iron and manganese (oxyhydr)oxides, with ferrihydrite, an iron oxyhydroxide, as a type example. Mineral nanoparticles are minerals that can also exist in larger sizes, and these probably include most of all known minerals.

Why do minerals exhibit a range of behaviors in the Earth environment as a function of particle size and shape when smaller than a few to perhaps as much as several tens of nanometers in at least one dimension? These variations are most likely due, at least in part, to differences in surface and near-surface atomic structure, as well as crystal shape and surface topography as a function of size in this smallest of size regimes. It has now been established that these variations may make a difference in important geochemical and biogeochemical reactions and kinetics. This recognition is broadening and enriching our view of how minerals influence the hydrosphere, pedosphere, biosphere, and atmosphere. Similarly, these results demonstrate that anthropogenic nanomaterials may have influences on natural environments that are beneficial, neutral, or deleterious depending on their exact chemistry, form, and environment, just as we see for minerals.

Elemental distribution and bioavailability, reaction pathways and catalysis, and mineral growth/solubility/weathering are all influenced in the natural environment by phenomena relevant to the nanoscale with no equivalent phenomena at scales larger or smaller. Dissolved ions in aqueous solution, versus that same ion in a 1 nm mineral, versus that ion in a 5 nm or larger mineral, all behave differently. Considering everything that passes through 200 or 2 nm filters as dissolved is not appropriate. Aqueous and gas-based reactions that occur on (or in conjunction with) a molecular cluster, versus on a small mineral nanoparticle of the same composition, versus on a 50 nm or larger mineral grain, again with the same composition, are predicted to most often show significantly different reaction pathways and kinetics. We predict that the same will be relevant with anthropogenic nanomaterials in Earth ecosystems.

Given the easily predicted complexity of many aspects of Earth-nanomaterial interactions, we believe that a gap will remain for some time between the commercial benefits of nanotechnology and an understanding of the potential risks of nanomaterials exposure to humans and the environment. Nevertheless, we know a great deal about material-ecosystem interaction from decades of past research, and a great deal about nanomaterials. This will provide an important foundation for the study of the environmental implications of nanotechnology in the near-term future.

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# **Synthesis, Size-Selective Separation, and Chemistry of Composite Synthetic and Natural Nanoparticles**

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With their high surface areas made up of chemically accessible atomic sites and their relatively low bulk lattice stabilization energies, nanoparticles are thermodynamically metastable entities that have the potential to readily interact with their molecular environment. In this context, they should also be viewed as transporters of atoms or electrons from their bulk lattice matrix as well as delivery agents for capping molecules or, in the case of porous nanoparticles, molecules intercalated in the nanoparticle pore structure. In this research we consider the low-temperature synthesis of nanoparticles and mesoporous nanospheres of redox active nanoparticles such as TiO<sub>2</sub> doped with Fe or Cu, which might be expected to modify cellular oxidation processes. The strategies used to create homogeneously doped, porous redox active nanoparticles with uniform nanoscale size distributions that form aqueous colloidal dispersions that are suitable for use in growth media will be described. The ubiquitous nature of layered clays and the wide variation in their surface charge properties, adsorptivities, and surface chemistries makes it impossible to ignore the role that they play in biological processes(1-3) and their consequent use as absorbants of pesticides(4-5), in medical applications(6), water purification(7-8), and radioactive(9) and heavy metal(10) cleanup, and as drug delivery agents(11) and catalysts(12-13). We will describe the size-selective separation, aqueous dispersion, and properties of four clay prototypes that have plate-like and fibrous morphologies and widely varying zeta potentials, ion exchange properties and chemical adsorptivities. The overarching goal of this research is to enable studies of how clay nanoparticles are altered in different environments, are transported between different media, serve to transport and mobilize chemicals, interact with cells, modify the environment around them, and how these processes affect their toxicity profile.

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# Molecular-Level Studies of Iron Oxide and Silver Nanoparticles – Structure and Reactivity

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Our work over the past year has focused on the structure and reactivity of (1) synthetic hematite ( $\alpha$ -Fe<sub>2</sub>O<sub>3</sub>) nanoparticles, (2) synthetic disordered “2-line” ferrihydrite nanoparticles, (3) synthetic ordered ferrimagnetic ferrihydrite nanoparticles, (4) natural and synthetic ferrihydrite nanoparticles containing up to 15.1 wt.% Al<sub>2</sub>O<sub>3</sub> and 9.1 wt.% SiO<sub>2</sub>, and (5) manufactured silver nanoparticles.

In our recent study of the structure and reactivity of hematite ( $\alpha$ -Fe<sub>2</sub>O<sub>3</sub>) nanoparticles, we made use of the interaction of Zn(II)<sub>aq</sub> with hematite nanoparticles (10.5 nm avg. diameter) and microparticles (550 nm avg. diameter) in the absence and presence of oxalate to assess differences in nanoparticle vs. microparticle surface structure and reactivity.<sup>1,2</sup> EXAFS spectroscopy, coupled with selective chemical extraction and attenuated total reflection FTIR spectroscopy methods, allowed us to detect significant differences in Zn sorption products on the two hematites of different particle size over a similar range of Zn surface loadings ( $\Gamma = 0.4$  to  $4.2$  mmol/m<sup>2</sup>), solution conditions (pH 5.5 and 0.01M background electrolyte), and reaction time (48 hr). For example, evidence was found for a (Zn,Fe)(OH)<sub>2(am)</sub> precipitate associated with the nanoparticles at  $\Gamma > 3.4$  mmol/m<sup>2</sup>, whereas no precipitates were detected in the microparticle systems at  $\Gamma \leq 4.2$  mmol/m<sup>2</sup>. Moreover, results from selective chemical extraction involving a CaCl<sub>2</sub> wash suggest that about 40% of the sorbed Zn(II) was present as more weakly bound outer-sphere complexes in the microparticle systems, whereas only 10-15% of the sorption complexes were outer sphere in the nanoparticle systems under similar conditions. In addition, analysis of Zn-Fe pair correlations for the two systems indicated that Zn(II) forms inner-sphere mononuclear bidentate, tetrahedral (Zn(O,OH)<sub>4</sub>) and octahedral (Zn(O,OH)<sub>6</sub>) surface complexes at low Zn surface coverages ( $\Gamma < 3.4$  mmol/m<sup>2</sup>), whereas only inner-sphere mononuclear bidentate edge-sharing octahedral Zn surface complexes were observed at  $\Gamma \geq 3.4$  mmol/m<sup>2</sup> for the nanoparticle systems and at all Zn sorption densities ( $\Gamma = 1.5$ - $4.2$  mmol/m<sup>2</sup>) in the microparticle systems. These observations led us

to conclude that the hematite nanoparticles are significantly more reactive than the microparticles. Geometric analysis of EXAFS-derived Zn-Fe 2<sup>nd</sup>-neighbor distances indicate that both 5- and 6-coordinated Fe<sup>3+</sup> occur at hematite nanoparticle surfaces, whereas only 6-coordinated Fe<sup>3+</sup> was detectable at hematite microparticle surfaces. These differences help rationalize reactivity differences of the hematite nanoparticles and microparticles. A similar approach is being used to study the reactivity of ferrihydrite and silver nanoparticles.

Ferrihydrite is one of the most important natural nanoparticles, occurring in a variety of natural environments. Our ongoing studies of the structure and reactivity of ferrihydrite have included total x-ray scattering and pair distribution function (PDF) analysis of ferrihydrite nanoparticles as a function of aging at 175°C in the presence of citrate, which slows the transformation of disordered ferrihydrite to hematite and reveals for the first time an intermediate phase that is larger in particle diameter (8-10 nm) relative to disordered ferrihydrite (2-4 nm) and is ferrimagnetic. PDF analysis showed that this intermediate has the same basic structure as recently proposed for disordered ferrihydrite<sup>3</sup>, but with fewer cation vacancies, fewer protons in the structural formula, and enhanced magnetism relative to disordered ferrihydrite. Attempts to fit the PDF with structural models similar to maghemite ( $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>) and goethite ( $\alpha$ -FeOOH) were unsuccessful. Thermogravimetric analysis data, coupled with the loss of protons accompanying the filling of Fe-site vacancies during aging, were used to constrain the chemical formulae of both disordered “2-line” ferrihydrite (Fe<sub>8.2</sub>O<sub>8.5</sub>(OH)<sub>7.4</sub>•3H<sub>2</sub>O) and the intermediate ordered ferrihydrite (Fe<sub>10</sub>O<sub>14</sub>(OH)<sub>2</sub>•~1H<sub>2</sub>O). PDF studies of natural and synthetic disordered ferrihydrites containing Si show that the size of the coherent scattering domain for Si-ferrhydrites are reduced relative to synthetic Si-free ferrihydrite. Little change in the ferrihydrite structure was noticed in the PDF's when Al is present. <sup>27</sup>Al NMR data coupled with scanning transmission x-ray spectromicroscopy indicate that Al is present in a separate phase, likely an Al-hydroxide, although some Al is also present in the ferrihydrite structure, which should affect its reactivity relative to pure ferrihydrite.

Although our work on silver nanoparticles is just getting underway, we have carried out total x-ray scattering and PDF analysis of PVP-coated Ag nanoparticles ranging in size from 5-50 nm and find that the face centered cubic structure and *a* cell parameter don't change significantly as a function of particle size. Exposure of the Ag nanoparticles to ambient air results in the formation of an acanthite (Ag<sub>2</sub>S) coating, as revealed by STXM and x-ray photoelectron spectroscopy.

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## Stability and photoactivity of metal oxide nanoparticles in natural waters

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Natural waters are a complex environment for nanoparticles (NPs), with significant differences in pH, ionic strength (IS), anions and cations, and natural organic matter (NOM). Mechanistic studies conducted by others at the UC CEIN have served to better understand the processes that are individually at play. However, it is just as important to determine the behavior of NPs in the more complex natural waters, where they will ultimately enter the environment. We conducted studies with seawater, a range of freshwater matrices, rainwater and wastewater, to determine the stability and photoactivity of three metal oxide NPs (ZnO, TiO<sub>2</sub>, CeO<sub>2</sub>) in these systems. The behavior is a balance between the stabilizing effect of NOM, the destabilizing effect of increasing IS, and the competing effects of monovalent vs. divalent cations, which alter the double-layer thickness surrounding the NPs. These behaviors play an important role in the bioavailability of the NPs, and their ultimately toxicity to different organisms within marine, freshwater and terrestrial exosystems.

# Surface interactions of thiol-containing organic acids on zinc sulfide and silver nanoparticles

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This work investigates the phenomena governing surface interactions between nanoparticles containing soft-sphere metals and naturally-occurring organic acids and describes the implications of these interactions for the fate and transport of the nanoparticles in natural aquatic systems. As described by Pearson's classification for metal-ligand binding, "soft" metals such as  $Zn^{2+}$ ,  $Ag^+$ , and  $Hg^{2+}$  have greater binding affinity for thiol-containing ligands over oxygen-containing ligands with hydroxyl and carboxylate groups. This study focuses on two types of nanoparticles containing soft-sphere metals: zinc sulfide (ZnS) and metallic silver ( $Ag^0$ ). ZnS particles occur naturally in anaerobic sediments and in surface waters located near sources of sulfides (e.g., wastewater discharge) [1, 2]. Engineered nanomaterials such as quantum dots can also contain ZnS coatings.  $Ag^0$  nanoparticles are widely used in various consumer products. Silver is known to be toxic to aquatic organisms in the ionic form ( $Ag^+$ ) [3]. The mechanism of toxicity by  $Ag^0$  nanoparticles has yet to be determined [4], but is likely to involve  $Ag^+$ . When nanoparticles of ZnS and  $Ag^0$  enter anaerobic sediments, surface waters and wastewater, natural organic acids may sorb to the particles and potentially alter the stability of the particles in aqueous suspension. Preferential binding by thiol groups associated with natural organic matter to the surface of ZnS and Ag is likely to occur.

In this study we investigated the surface sorption of two amino acids, cysteine and serine, on ZnS and  $Ag^0$  nanoparticles and the effect of this interaction on particle aggregation rates. These organic acids were chosen for their prevalence in the aquatic environment and in biological systems. These amino acids were also chosen to compare ligands that are structurally similar except for substitution of one functional group (thiol in cysteine versus hydroxyl in serine). Dynamic light scattering was used to assess the effects of these organics on the homogeneous aggregation rate of ZnS nanoparticles under a range of solution conditions (pH, monovalent electrolyte concentration, and organic acid concentration). The results indicated that the presence of serine did not alter ZnS aggregation rates relative to the amino acid-free control. In contrast, cysteine reduced ZnS aggregation rates by orders of magnitude, depending on the concentration of cysteine, ionic strength and pH. The changes to colloidal stability were a result of organic-particle surface interactions, since cysteine was found to adsorb to ZnS nanoparticles. Serine did not adsorb to the particles. Furthermore, evaluation of the cysteine-coated ZnS nanoparticles by zinc X-ray absorption near edge spectroscopy (XANES) indicated that surface Zn atoms were binding to sulfhydryl groups associated

with cysteine. In experiments with PVP-coated silver nanoparticles, cysteine adsorbed to the material, indicating the presence of oxidized Ag(I) on the particle surface. Cysteine also increased the concentration of dissolved silver in the nano-Ag<sup>0</sup> suspension, demonstrating that ligand-promoted dissolution of the particles was occurring. These results highlight the importance of metal-ligand interactions for controlling surface association of natural organics on nanoparticles. Surface sorption with cysteine, humic substances, and other thiol-containing acids can influence colloidal stability and dissolution of the nanoparticles in porewater and within cell tissue.

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# Schwertmannite: A natural nanomaterial's role in arsenic cycling

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One of the goals of the Center for the Environmental Implications of NanoTechnology (CEINT) is to compare and contrast the behavior of natural and engineered nanomaterials. As defined in a recent *Science* paper (1), schwertmannite is an example of a natural nanomineral. Nanominerals “show variations in their atomic structure relative to larger particles and as a function of size in the nanoscale regime” (1). Our group studies the unique role that nanominerals play in Earth processes because of their small size. Recent studies have shown (2,3) that schwertmannite plays a key role in the retention of arsenic in acid mine drainage sites. Due to their unique properties of increased and highly reactive surface area, engineered nanomaterials are also used to remediate toxic metals like arsenic (4). Our research project is designed to identify the fundamental properties underlying schwertmannite's ability to retain arsenic, giving a basis for comparison with engineered nanomaterials of similar type.

Schwertmannite is a little-understood iron oxyhydroxide sulfate nanomineral that is commonly formed in the presence of Fe- and sulfate-enriched waters in a pH range from 2 to 4, but due to its poor crystallinity, metastability, and small size, it has been “viewed with caution” and only recognized as a distinct mineral since 1994 (5). We predict that variations in schwertmannite's surface and near-surface atomic structure can drive its reactivity and, in particular, its retention of the toxic metal arsenic. Furthermore, schwertmannite's metastability with respect to goethite – a more crystalline iron oxide – will further influence the fate of arsenic in these systems as it is unclear as to whether or not arsenic will be retained after transformation to goethite.

In the first phase of our investigation we have synthesized schwertmannite under various temperatures and reaction times. We have also reproduced the transformation of schwertmannite to goethite and showed that this process is time and temperature dependent. Using these syntheses we obtained schwertmannite with varying morphology (Figure 1) as well as intermediate forms of schwertmannite to goethite transformations. Through a combination of arsenic sorption experiments and characterization with a FEI

Titan 300 scanning transmission electron microscope, we will reveal the fundamental properties governing schwertmannite's role in arsenic cycling.

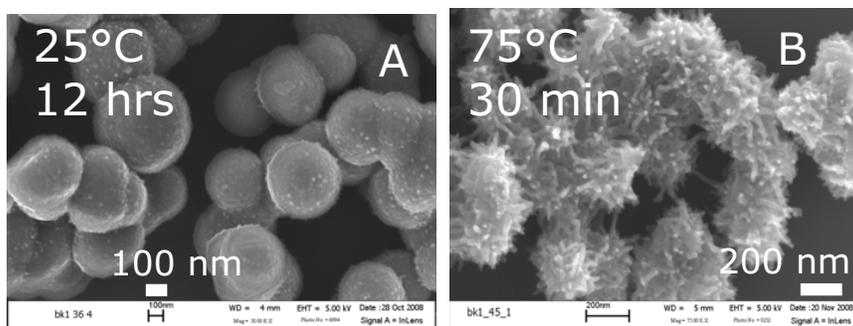


Figure 1. (A) Smoother schwertmannite spheres are produced at lower temperatures, whereas in (B) higher temperatures produce a rougher surface with spikes of only 5 nm in diameter.

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## Session 5: Nanomaterial Characterization & Toxicity

Co-Chairs: Jie Liu and Ponisseril Somasundaran

Thursday, September 10, 2009

9:00 a.m. – 12:20 p.m.

Forum 144/146

## **Challenges and current state of physicochemical characterization and detection of nanoparticles in environmental media**

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### **Abstract**

To ensure sustainable development of nanotechnology, risk assessments of engineered nanoparticles (ENP) from various applications are required and for that it is essential to have the tools to carry out both effect and exposure assessment. That requires thorough physicochemical characterization of nanoparticles and their aggregates. The characterization needs include initial detailed material characterization and dispersion properties and exposure characterization during effect experiments.

There is a range of characterization methods available to the field of nano(eco)toxicology, that can determine relevant phys-chem properties, each with its own advantages and limitations<sup>1-5</sup>. Thus we need to agree on a suite of complementary techniques. Some harmonization and standardization of instrumentation selection and method optimization is important to produce comparable and reproducible results. Here are discussed method validations in relation to analytical chemistry, particle size analysis in ceramic and powder science, as well as nanometrology, and some suggestions for methods will be given.

Conventionally, exposure assessment is recommended to include both a modeling and a measurement approach, and both approaches require instrumentation and methodology of nanoparticle analysis. Prediction of environmental concentrations through modeling is mainly founded on emission scenarios and distribution parameters (fate and behavior). Presently, fate and behavior parameters of ENP are not known, and use of suitable analytical methods to determine the concentrations and nanoparticle characteristics in lab and field experiments is a prerequisite to acquire such distribution parameters. As the complementing approach to modeling, it has been emphasized that one of the main challenges for safe implementation of nanotechnology is the development of methods to monitor nanoparticles in the environment.

The main challenges for detection and characterization of engineered nanoparticles in aquatic environmental samples are that there are extremely few engineered nanoparticles but plentiful of natural or unintentionally produced nanoparticles. This high background of nanoparticles renders otherwise powerful microscopic techniques (e.g. TEM, AFM) unpractical. This is because they need to screen millions or billions of background nanoparticles in order to have measured and characterized enough ENP.

Our aim is to develop an analytical method with low enough detection limits and high enough selectivity to enable single nanoparticle detection and analysis in size fractionated aqueous samples. Initial experiments from Field-Flow Fractionation (FFF) coupled to ICPMS in an ultra fast scanning mode, show promising preliminary results that the method can be developed to meet the required capabilities. Results will be shown for engineered nanoparticles in highway runoff water and waste water effluent samples.

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## **Analytical Characterization of Silver Nanoparticles and Nanoparticle Doped Media**

James Mitchell, Kyethann Francis, Andy Hai Ting

Howard University, Washington, DC 20059

Accurate conclusions about the impact of nanoparticles within the environment of microbes to man depend unequivocally on the reliability of information on the initial chemical and physical status of the nanomatter, and its time dependant transformation within the environment. The ultimate impact potential may further depend on vanishingly low levels of concentration, and the complexity of physicochemical interactions within natural environments, and even more specifically within controlled laboratory settings. Additionally, researchers scrutinizing the impact of nanoparticles within the environment must cope with challenges presented by increasing potential for adventitious contamination, and losses during the processing and handling of nano-sized materials in aqueous cellular and microbial media. Thus characterization science and metrology of submicron-sized particulates are vitally important, and methodologies for the proper chemical handling and processing of nanomaterials are essential for reliable nanoenvironmental research.

In this presentation the authors examine systematically the various aspects of preparing silver citrate terminated nanoparticles, and verifying their chemical and physical stability in pristine aqueous media, and subsequently in cell culture media widely utilized to research the cytotoxic or intracellular impact of environmental constituents on microbes and cells. It is demonstrated that the plasmon resonance absorption of silver nanoparticles (Figure 1) provides a viable probe for ascertaining the chemical and physical stability of the nanoparticles. Both the shape of the spectra (Figures 1 & 3) and linear dependence with dilution (Figure 2), when coupled with constancy of sized with time (Figure 4), provide reliable means of confirming the time dependant duration of the adequacy of protocols for the quality assurance of experimental results.

Exact procedures for determining the shelf-life of nanoparticle reagents, degradation during cold storage, and deterioration of doped media are presented. These characterizations are demonstrated to be executed most sensitively by resonant light scattering (Figure 5). Application of the method provides examples documenting the invalidation of experimental results and conclusions in the absence of simultaneous execution of reliable analytical chemistry. Also, approaches to the assurance of the absence of by-products and chemical impurities, and unpredictable chemical reactions of the doped nanoparticles with components of cellular media are examined. The full array of potential chemical components of silver nanoparticles generated in aqueous media is discussed. Selective spectrophotometry, thermometric titrations, and zeta potential characterizations provide supporting information.

## High throughput multiparametric cytotoxicity screening for predictive toxicological assessment of nanomaterials

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Towards the realization of a verifiably safe nanotechnology, we report a high throughput toxicity screening based on hierarchical oxidative stress paradigms and materials re-design to ameliorate the hazardous characteristics of zinc oxide (ZnO) nanoparticles. The hazard assessment of ten different nanoparticles at multiple doses and durations in bronchial epithelial and macrophage cell lines were carried out by employing a combination of fluorescent dyes that can be loaded contemporaneously. An integrated series of cytotoxic effects were quantified by automated epifluorescence microscopy. Nanoparticles exhibited differential toxicity pattern as evaluated by cytotoxic effects such as mitochondrial superoxide generation, mitochondrial depolarization, increased intracellular calcium flux, and surface membrane leakage. As an example of purposeful amelioration of toxicity based on mechanistic understanding, we report the reduction in toxicity of ZnO nanoparticles achieved by doping with iron. Doping of ZnO with incremental concentrations of iron reduced the dissolution of Zn<sup>2+</sup> ions, consequently reducing the toxicity in a concentration dependent manner. Hence, by combining *in vitro* detection of a hazardous nanomaterial property that can be correlated to *in vivo* toxicity and safe material design, this work exemplifies a rational approach towards safer nanotechnology applications.

## **Analysis of single-walled carbon nanotubes in estuarine sediments by near infrared fluorescence spectroscopy**

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Single-walled carbon nanotubes (SWNT) have emerged as a promising material for commercial and industrial applications. It is clear that as these nanomaterials become more commonplace, they will eventually reach the ambient environment through waste discharge or disposal. Our recent work and that of others has shown that SWNT have high affinity for natural particulates in aquatic systems and are thus expected to concentrate in sediments after discharge to receiving waters. Any assessment of the occurrence and fate of SWNT in the aquatic environment will thus necessitate development of sensitive and selective detection of these materials in sediments. Near Infrared fluorescence (NIRF) spectroscopy has advanced as a highly selective and information-rich technique for sensitive detection and structural characterization of SWNT materials. We report here the development and validation of a novel analytical technique based on NIRF spectroscopy for quantitation of SWNT in estuarine sediments. SWNT tend to aggregate into bundles in aqueous solution due to strong van der Waals attraction, and this effect tends to reduce the quantum yield of SWNT, reducing NIRF sensitivity. Extraction of SWNT from sediments into surfactant solutions allows reduction of sample complexity and enhanced disaggregation of bundled SWNT prior to NIRF analysis. We have tested multiple surfactants singly and in mixtures for their ability to extract and disaggregate SWNT from sediments prior to NIRF analysis. CoMoCAT nanotubes were extracted from nanotube-spiked sediments by ultrasonication in 2% surfactant solutions and individual surfactant-wrapped nanotubes were isolated from aggregates by ultracentrifugation. Results reveal that sodium deoxycholate was the most effective surfactant for extraction of SWNT from sediment and subsequent exfoliation prior to NIRF spectroscopic analysis. SWNT extracted from sediment in 2% sodium deoxycholate could be quantified down to 9 ng/mL, and detection was linear over > 3 orders of magnitude.

# Stability of Silver Nanoparticles under the Irradiation of Sunlight

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The outstanding properties of silver nanoparticles (Ag NPs) have made them incorporated in many commercial products (1). For example, they are used in socks to restrict the growth of odor-causing bacteria. Such wide application has raised the concern on the fate of Ag NPs in natural environment once they got released. Unfortunately, little is known in this area.

In this project, we studied the fate of Ag NPs under the irradiation of natural sunlight. Two kinds of Ag NPs that we used were GA-coated ~25nm and PVP coated ~20nm Ag NPs. These nanoparticles were freshly prepared and purified by centrifuging three times at 15000 rpm ( $F=27000g$ ) for 1h, followed by re-dispersing in nanopure water. The final Ag NPs dispersions were placed under natural sunlight (Figure 1), with the corresponding control samples kept under laboratory condition. The fate of nanoparticles was tracked by UV-vis, TEM and ICP.



Figure 1 Experimental setup of the study of the effect of sunlight irradiation on the stability of silver nanoparticles.

Under sunlight irradiation, the PVP coated and GA coated Ag NPs undergo different degrees of aggregation. For the PVP coated Ag NPs, the UV-Vis spectrum (Figure 2A) indicates that the characteristic absorption peak of Ag NPs around 400nm (2) decreased and another peak at higher wavelength, appeared and gradually red shifted, indicating the formation of anisotropic nanomaterial, with gradually increased aspect ratio. This is also supported by TEM. Figure 3A is the initial TEM image of these Ag NPs, showing that they were spherical in shape and uniform in diameter. But after 7 days of sunlight irradiation, they aggregated and linked together, forming chain-like structures. Nevertheless, the silver concentration remained constant within the solution (Figure 1B), suggesting that they still stable in the solution.

For the GA-coated Ag NPs, the UV-Vis spectrum (2C) shows that the characteristic adsorption peak of Ag NPs around 400nm is gradually decreased and disappeared after 4

days. The ICP results suggest the silver concentration in the solution decreased dramatically within 4 days. The observation of visible solid at the bottom of vial implies that the Ag NPs have precipitated out from the solution. The TEM images of GA coated Ag NPs (Figure 3C and 3D) indicate that the initial Ag NPs are also spherical and uniform, but after 4 days of sunlight irradiation, the Ag NPs aggregated seriously and formed much bigger particles, to the point that they are no longer stable in solution.

In conclusion, we have studied the effect of sunlight irradiation on the stability of Ag NPs. We found that both PVP coated and GA coated undergoes certain degrees of aggregation. The PVP coated Ag NPs were linked but were still stable in solution within 7 days, on the other hand, the GA coated Ag NPs experienced serious aggregation and precipitated out from the solution, leading to dramatic decrease in silver concentration in the solution. It is suspected that the UV component of sunlight is responsible for such change (with data not shown here).

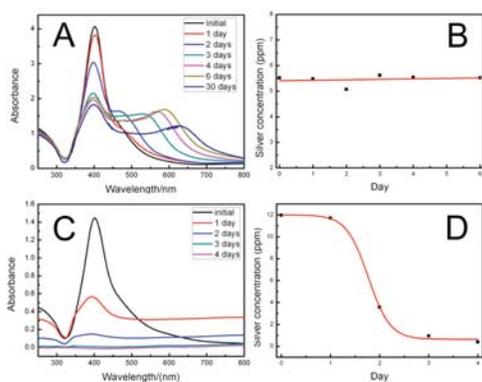


Figure 2 Evolution of (A) UV-Vis absorption spectrum and (B) concentration of silver of PVP coated Ag NPs under the irradiation of natural sunlight; Evolution of (C) UV-Vis absorption spectrum and (D) concentration of silver of GA coated Ag NPs under the irradiation of natural sunlight.

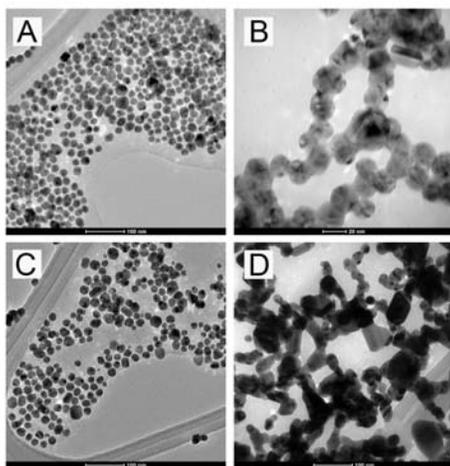


Figure 3 TEM images of (A) initial and (B) after 7 days of sunlight irradiation of PVP coated Ag NPs; (C) initial and (D) after 3 days of sunlight irradiation of GA coated Ag NPs.

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**Correlation of wettability/hydrophobicity of nanoparticles with their toxicity on  
*Nitrosomonas europaea*  
Ponisseril Somasundaran\* and Xiaohua Fang**

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Nanoparticles are increasingly used for a variety of purposes with reports on their toxicity also appearing in larger numbers. The wettability/hydrophobicity is an important property of nanoparticles that determines their engulfment in cells and transport and fate in the environment. Currently, however, there is barely any reliable way to characterize such a property down to the nano scale. We show here a novel technique developed to quantify such a property. It is based on monitoring the evaporation of liquid with and without the presence of nanoparticles. Compared to the traditional method, this method is easy, accurate, and robust. The hydrophobicity grading of the various fine particles is therefore feasible, which is important for predicting the resultant potential nanotoxicity, as shown by the experimental results of interaction of titania, zinc oxide and cerium oxide particles with *Nitrosomonas europaea*.

# Optimization of Nanoparticle Dispersion in Various Cell Culture Media

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Characterization of nanoparticle size and state of dispersion in biological environments and understanding the parameters that affect them are imperative to accurately assess nanoparticle toxicity. In the case of *in vitro* studies, nanoparticles must be well-dispersed in order to uniformly dose the cells. Similarly, for *in vivo* studies, delivering nanoparticles in a well-dispersed form is necessary for accurate assessment of their toxicity. Based on these considerations, the main focus of the present investigation is to accurately evaluate the particle sizes of a wide range of nanoparticles, including metals, metal oxides, semiconductor quantum dots, and carbon nanotubes, in water and various cell culture media. To improve the dispersion of these nanoparticles, different dispersing agents such as bovine serum albumin (BSA), fetal bovine serum (FBS), dipalmitoylphosphatidylcholine (DPPC), and alginic acid sodium salt (alginate) were explored and the concentrations of these agents were optimized. The preliminary results obtained using high throughput dynamic light scattering (DLS) suggest that the nanoparticle agglomeration was effectively reduced upon addition of these dispersing agents in the cell culture media. The best dispersion was achieved when FBS was used as the dispersing agent. Addition of 1 wt.% FBS led to highly stable suspensions with nanoparticle concentrations up to 100  $\mu\text{g mL}^{-1}$ .

# UCLA Molecular Shared Screening Resource: Capabilities and Applications for Nanotoxicity Studies

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The central goal of the UC Center for Environmental Implications of Nanotechnology (UC CEIN) is to develop a broad-based model of predictive ecotoxicology of nanomaterials (NM) premised on quantitative structure–activity relationships (QSARs) and NM injury mechanisms at the biological level. Establishing a predictive science is a timely approach for nanotechnology-based enterprises wishing to avoid the problems faced by the chemical industry, where only a few hundred of the *ca.* 40,000 industrial chemicals have undergone toxicity testing, making it very challenging to control the toxicological impact of chemicals in the environment. A recent report by the National Research Council of the National Academy of Sciences set forth a vision of dramatic change in toxicological testing from individual testing to a predictive high-throughput paradigm premised on the established mechanisms and pathways of toxicity. Within the UC CEIN, we are using high throughput screening (HTS) to develop a rich database of toxicological outcomes for nanomaterials that provides the large volumes of data needed to develop a robust predictive model and also allows us to prioritize which materials should be the focus of more in-depth low-throughput studies at the organismal and population level.

The hallmarks of HTS are high sample throughput, miniaturization, and full automation of the assay process. This approach requires robotics, data processing and control software, liquid handling devices, and sensitive detectors. Although developed in the pharmaceutical industry, HTS is not limited to drug discovery: it can be used to quickly conduct thousands of tests for any assay that is amenable to automation. We are using existing HTS capabilities at the UCLA Molecular Screening Shared Resource (MSSR) to rapidly determine the specific features of NMs that govern their biological interfacial properties, environmental fate, and transport.

Established in 2003, the MSSR provides HTS technology. Since 2005, it has been

directed by Ken Bradley and Robert Damoiseaux, who report to an Advisory Board with members from UCLA's CNSI, Chemistry, Biology, Medicine, and other departments. The MSSR contains two fully integrated systems: (i) Automated liquid handling, multiple plate reading, plate filling and washing, deshiielding, and delidding, and online incubators for cell-based assays using a Beckman/Sagian system equipped with an Orca robotic arm that delivers plates to individual work stations. Current capacity of cell-based assay is ca.  $10^5$  wells (conditions)/day. Multiple plate readers allow fluorescence, FRET, BRET, time-resolved fluorescence, fluorescence polarization, luminescence, and UV-Vis absorption assays. (ii) A second Beckman/Sagian Core system for HCS using automated microscopy with an Orca arm and a Molecular Devices ImageXpress (micro) automated fluorescence microscope and a Cytomat 6001 incubator. Additional equipment in the MSSR that is available for off-line use includes a Genetix Q-bot colony-picking robot to maintain and re-order clone collections; a Hydra 96 and Precision 2000 automated pipetting and manifold; 6-ft cell culture facilities, molecular biology capabilities,. MSSR screening capabilities include two genome-wide knockout libraries of *S. cerevisiae* yeast, a genome-wide knockout library of *E. coli*, and genome-wide small interfering RNA libraries for mouse and human, providing functional genomic capabilities for identifying cellular pathways governing responses to nanomaterials. Here, we discuss how these robotic systems and libraries can be used for toxicological screening, using examples of systems being studied in the UC CEIN.

Session 6: Ecology & Ecosystem Response

Co-Chairs: Paul Bertsch and Bradley Cardinale

Thursday, September 10, 2009

9:00 a.m. – 12:20 p.m.

Auditorium 151/155

# **Known Knowns, Unknown Knowns and Unknown Unknowns: Challenges to Understanding the Impacts of Nanomaterials in Ecosystems**

**Emily Bernhardt<sup>1</sup> and Joshua Schimel<sup>2</sup>**

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<sup>2</sup>UC Santa Barbara, Santa Barbara, CA.

Predicting the ecosystem impacts of nanomaterials requires significant progress in understanding 1) abiotic interactions between nanomaterials and natural substrates and solutions; 2) interactions between nanomaterials and ecosystem primary uptake compartments (plants, fungi and bacteria); 3) the resulting consequences of nanomaterial exposure for productivity, organic matter decomposition and trophic transfer. To date there has been little empirical research conducted on any of these issues either individually or collectively, thus current predictions of ecosystem impacts are derived from simplistic assumptions about nanomaterial surface chemistry and reactivity together with limited ecotoxicological information. Such “lab-scale” information may be misleading – and pure culture studies in the lab often find higher toxicity than the limited field studies conducted to date. Ultimately we need a mechanistic understanding of the forms in which nanomaterials are transported through and retained within organisms and abiotic ecosystem compartments so that we can make “bottom-up” predictions about the likely impacts of nanomaterials on biological populations, communities and ecosystems. Concurrent, “top-down” research efforts are required that measure the ecosystem scale responses to nanomaterial exposure. It is these ecosystem scale experiments that can conclusively demonstrate likely environmental impacts, as well as provide important guidance for prioritizing the most critical first-order, mechanistic research questions that need to be addressed.

# **Differential effects of nano-particulate Zinc Oxide and Titanium Dioxide on the population growth rates of marine phytoplankton**

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Metal oxide nanoparticles are an emerging class of pollutants that may affect aquatic ecosystems, including coastal marine habitats. Phytoplankton dominate the base of the food web in most marine habitats, and their growth rates, primary production levels, and species composition can profoundly affect the entire ecosystem. Using standard reference particles characterized by UC-CEIN, we tested the effects of two types of metal oxide nanoparticles, zinc oxide and titanium dioxide, on representatives of three major phytoplankton groups: diatoms (*Thalassiosira pseudonana* and *Skeletonema costatum*), prymnesiophytes (*Isochrysis galbana*) and chlorophytes (*Dunaliella tertiolecta*). Our results show that these particles can have a significant negative effect on population growth rates of marine phytoplankton, but that effects differ depending on particle type.

# Microbial Inhibition and Silver Resistance Development in Wastewater Containing Silver Nanoparticles

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Silver nanoparticles are increasingly being used mostly because of their beneficial antimicrobial properties. They have been incorporated into a wide range of consumer products, including wound dressings, air filters, washing machines, textiles, and baby pacifiers (1). However, the potential ecological impacts stemming from their environmental release are unclear. It is possible that silver nanoparticles could alter and even negatively impact microbial communities in a variety of ecosystems. Polyvinyl alcohol coated silver nanoparticles have been linked to a decrease of nitrifying activity in mixed nitrifying bacterial communities commonly found in municipal wastewater treatment plants, which is a key step in nitrogen removal from wastewater (2). Furthermore, it is likely that, with repeated exposure to silver nanoparticles, microbial communities could evolve and develop resistance to silver. Thus, a long term effect of silver nanoparticle exposure could be a reduction of the efficacy of such products in a similar fashion to the development of microbial antibiotic resistance (3). Therefore, it is critical that the ecological impacts of these materials be ascertained in both engineered and natural environments to prevent long term negative effects.

The objectives of this study are two-fold. First, microbial growth of mixed communities originating from activated sludge was determined in the presence of silver nanoparticles with a range of sizes and coatings. Second, microbial adaptation in terms of silver resistance development was investigated. All experiments were conducted in 150 mL batch reactors containing 100 mL synthetic wastewater, seeded with 1% activated sludge obtained from the North Durham wastewater treatment facility (Durham, NC). Three different silver nanoparticle coatings were tested (citrate, gum arabic and PVP) in concentrations ranging from 0.2 to 200 mg/L. Silver nitrate was used as the positive control while reactors containing either no silver or sodium azide inactivated sludge were used as the negative controls. All samples were prepared in triplicate. Optical density was measured daily by spectrophotometry to monitor microbial growth. DNA was extracted at the beginning and end of each experiment for community structure analyses. Total dissolved silver concentrations were monitored by ICP-AES.

Experimental data have shown that silver nanoparticles delay and inhibit microbial growth of activated sludge microorganisms. The degree of inhibition appears to be linked to the total dissolved silver concentrations in the media. These results suggest that silver nanoparticles can be just as potent growth inhibitors as ionic silver. Further studies using

ICP-AES are being conducted to quantify the amount of dissolved silver produced by the addition of nanosilver. Efforts are underway to determine the relationship between microbial inhibition and dissolved silver concentration.

Finally, experiments are currently being performed to quantify microorganisms carrying two silver resistance genes (*silS* and *silR*) in the batch reactors previously described. The persistence of these genes is well established in humans and animals resistant to silver treatments (4,5), however there has been little focus on studying their prevalence in wastewater systems. Characterizing the prevalence of silver resistant microorganisms in wastewater communities is the first step towards understanding the ecological implications of discharging products containing silver nanoparticles into wastewater.

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## **TiO<sub>2</sub> nanoparticles stimulate biomass production in freshwater algae**

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As improvements in technology have led to an explosion in the production and use of nanomaterials, parallel efforts have been made to understand the unintended biological impacts of nanomaterials on everything from cellular metabolism to the productivity and sustainability of ecosystems. Algal biomass production is one of the most fundamentally important ecological processes on the planet, and is largely responsible for controlling the flux of key gases like O<sub>2</sub> and CO<sub>2</sub> to and from the atmosphere, and controls the quality of water in streams, lakes and oceans. Here we report the results of a laboratory experiment in which we examined how TiO<sub>2</sub> nanoparticles impact the production of biomass by freshwater algae. We exposed 20 of the most common species of North American algae to five increasing concentrations of TiO<sub>2</sub> (ranging from 0-ppm controls to 300-ppm), and measured growth rates and biomass production over a period equivalent to 10 generations. While the responses of algae to increasing levels of TiO<sub>2</sub> varied among species, the growth of most species (75%) was stimulated by the presence of TiO<sub>2</sub>. Compared to controls, growth rates were between 1.5 and 3x higher, and maximum biomass was often an order of magnitude higher in experimental units containing TiO<sub>2</sub>. The biological mechanism(s) producing these responses is(are) uncertain. We are working to verify or eliminate the potential for TiO<sub>2</sub> to (i) stimulate photosynthesis through photo-activation, (ii) increase nutrient availability through degradation of organic matter, (iii) alter competitive interactions between algae and bacteria by differentially depressing the metabolism of prokaryotic cells, and/or (iv) provide additional growth substrates on aggregates. Regardless of what mechanism is ultimately supported, our results suggest that nano-sized particles of this metal oxide can stimulate the production of algae in streams and lakes.

# **The Impact of Reactive Iron Nanoparticles on Microbial Diversity in Aquifer Soils**

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Nanotechnology has the potential to revolutionize how the world addresses many challenging problems, from drug delivery to groundwater remediation. These useful emerging technologies need to be thoroughly evaluated to determine if any there will be any unforeseen negative consequences to health or the environment when the technology is implemented on a large scale. This project examines the impact of reactive nanoparticles, designed for the remediation of chlorinated solvent contaminated aquifers, on the native aquifer microbial populations.

Nanoscale zero valent iron particles (NZVI) are promising for use in the remediation of trichloroethylene (TCE) contaminated aquifers. Their small size allows the particles to fit through pores in the ground, and the high surface area allows the particles to rapidly react with TCE to reduce it to the nontoxic products ethene and acetylene. The surface of the particle can be modified with an anionic polyelectrolyte to aid in particle transport. This study used microcosms of soil from three different TCE contaminated sites to examine the impact of NZVI on geochemistry and microbial diversity. The addition of NZVI rapidly created reducing conditions favorable for sulfate reducing bacteria and methanogens. Although nanoparticles (zero valent iron nanoparticles in particular) have been shown to be toxic to bacteria in suspended cultures, no negative impact on the overall number of bacteria was observed in the soil systems. Bacterial populations even increased when polyaspartate modified NZVI was added to the system. The lack of an observed toxic effect has positive implications for field scale application of NZVI.

# **Physicochemical and biological interactions between manufactured nanoparticles and environmental bacteria.**

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Thanks to their particular properties due to their size, the nanomaterials (dimension < 100 nm) are widely used in many industrial applications (daily care products, nanostructured materials...). However, their growing use frightens because of their uncertain impact on humans and environment. This study is dedicated to a deep understanding of the physicochemical and biological interactions between two cellular models from the environment: *Synechocystis* (cyanobacteria essential for the biosphere) and *Escherichia coli* (bacteria of mammalian intestines) with cerium oxide nanoparticles (ex: diesel additive).

The complexity of the study of nanoparticles requires a different approach (multidisciplinary) from those of toxicity tests of classic compounds. Indeed, we showed that the physicochemical parameters (stability, aggregation, dissolution and surface chemistry) of nanoparticles in the contact medium, strongly influence the toxicity (1). Furthermore, the physicochemical interactions (flocculation, adsorption, redox mechanisms) are linked to the biological model and especially the presence of exopolysaccharides (for *Synechocystis*) as natural barrier between the cell wall and the nanoparticles. Moreover, the composition of the nanoparticles dispersion medium (particularly the pH) has a major influence on the toxicity (survival and membrane integrity), whereas for *E. coli*, the nanoparticles are the main culprits for the mortality (confirmed by metabonomic studies via NMR) (2).

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Analytical approaches for in situ characterization of metal and metal oxide nanoparticles in soil ecosystems.

Jason Unrine, Aaron Shoults-Wilson, Jonathan Judy, Olga V Tsyusko and Paul Bertsch.

Detection and analysis of nanoparticles in complex matrices remains as one of the key challenges, not only for regulating nanomaterials in the environment, but also for studying their fate, transport and toxicity. Electron microscopy (EM) has been used heavily and can be a powerful tool, but there are limitations to EM based techniques including tedious sample preparation, poor counting statistics, and invasive analytical conditions. We are exploring a variety of advanced analytical approaches for investigating the spatial distribution, chemical speciation and speciation transformations of manufactured nanoparticles in soil ecosystems. Ecosystem components under investigation include soil invertebrates and plants, soil pore-water and leachates. One promising online technique under development is asymmetrical flow field flow fractionation (AF4) separation coupled to a variety of online detectors (i.e. multidetection). The detection methods that we have utilized include static and dynamic light scattering, UV-Vis spectroscopy, refractive index detection and inductively coupled plasma mass spectrometry. We will highlight AF4 based techniques to characterize nanoparticle binding to proteins in biological samples. We will also highlight applications of synchrotron based x-ray microspectroscopy and laser ablation-ICP-MS; which can be useful for examining the spatial distribution and chemical speciation of metal and metal oxide nanomaterials. Our studies have demonstrated the need to apply a variety of characterization techniques, each of which has strengths and weaknesses, to obtain a more complete picture of nanoparticle behavior in ecosystems.

Bioaccumulation and biomagnification of CdSe Quantum Dots in a simplified microbial food chain

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Engineered nanomaterials (ENMs) are entering the environment in unknown but increasing rates and quantities. ENMs come in a variety of shapes and compositions and are used in a wide range of products, from medical therapies to consumer products. Potential entry routes of ENMs into the environment range from the flaking of paints, disposal of products in landfills, production exhaust, and even leaching from sunscreens and cosmetics. ENMs have been shown to disrupt cellular membranes, induce ROS production and kill both prokaryotic and eukaryotic organisms. Because ENMs are able to enter and accumulate inside of living cells, they have the potential to be transferred between trophic levels. To understand the potential for ENMs to biomagnify up the food web we have investigated the transfer of CdSe quantum dots (QDs) from bacteria to a protozoan predator. Previous work on CdSe QDs has shown that they impair the growth of, induce ROS, and accumulate in *Pseudomonas aeruginosa* strain PG201(1).

*Tetrahymena thermophila* is a consummate bacterivore. It is a freshwater member of the ciliated protozoa, a major eukaryotic evolutionary group comprising common inhabitants of all aquatic environments. We used *Tetrahymena thermophila* strain SB210E as an apex predator in a mini food chain. PG201 cells were grown in the presence of QDs, Cd(II) or without any metals (control). At the concentrations used, PG201 cells were able to accumulate Cd(II) and the QDs while continuing to grow and divide. The three types of PG201 cultures were washed free of QDs and Cd(II) and were then separately fed to SB210E cells. We tested for toxicity, measured depredation rates of PG201 cells, SB210E division rates, and concentrations of the QDs and free Cd(II) in cells of both species. (Se, at equivalent concentrations, has no toxic effects on either

PG201 or SB210E cells and was not followed in these experiments.) EM observations provided information on the intracellular fate and integrity of the QDs.

Our results showed clear evidence of toxicity of both the QDs and the free Cd(II). SB210E cells readily consumed PG201 cells and grew at control rates in all treatments for 4 hours. However, by hour 6, feeding and growth had ceased in both the QD and Cd(II) treated cultures. By hour 16, all SB210E in both treated conditions showed morphological abnormalities under Nomarski optics and were dead (but not lysed), while the control culture continued to divide at the normal rate. EM observations showed that during hours 1 to 4, QDs were distributed unequally within the SB210E cells, with higher concentrations present in the mitochondria and in several pockets near the cell membrane as well as in membrane bound vesicles just outside the cell membrane. By hour 24 QDs were present throughout the SB210E with only slightly higher concentrations in the mitochondria. A key finding from this experiment was that the QDs remained predominantly intact throughout the experiment. Our calculations lead us to conclude that, on a Cd basis, the trophic transfer of QDs and Cd(II) from the bacteria to the protozoa was accompanied by a 784-fold QD biomagnification and a 747-fold Cd(II) biomagnification. Since the Tetrahymena cells remained physically intact, the potential appears to exist for additional biomagnification and toxicity in a more complex ecosystem.

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Poster Session

Wednesday, September 9, 2009

5:00 p.m. – 7:00 p.m.

West Ballroom

# Current Practices and Perceived Risks Related to Health, Safety and Environmental Stewardship in Nanomaterials Industries

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Environmental health and safety (EHS) and product stewardship practices specific to engineered nanomaterials (ENMs) are still being developed. Without regulation, ENM industries may act independently to avoid risks, and risk perceptions could profoundly influence industry practices. In 2006, an interdisciplinary team at the University of California Santa Barbara (UCSB), with support from the International Council on Nanotechnology (ICON), surveyed ENM industries worldwide regarding workplace and environmental practices, product stewardship, and risk perceptions (1). Since 2006, the ENM industry population has expanded, potentially resulting in changing views on risk and associated practices. This project aims to understand what steps ENM firms--producers and users of ENMs--are currently taking to protect workers, consumers, and the environment. We ask: 1) In the context of absent regulation and indeterminate standards, how is industry adapting their practices for the safe development of nanomaterials? 2) What do nanotech industries believe to be the roles of government(s) and private industry in ensuring the safe development of nanotechnology? 3) What determines the extent to which ENM firms follow publically-available guidance documents on nano-specific health and safety practices? 4) What are the unmet knowledge and guidance needs of industry? The project focuses on understanding the relative influence of *risk perception* and *regulatory frameworks* on *risk management* practices within the context of uncertain risk. This research applies a survey instrument developed through collaboration between social and natural science researchers. Researchers will elicit responses from an international pool of industry participants for a cross-national comparative analysis.

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# UV irradiation of nanoparticles in simulated natural water systems

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Very little is known about the environmental chemistry of nanomaterials (NMs). The generation of reactive oxygen species (ROS) and the quenching of ROS are two important reactions in natural aqueous systems where NMs may have an impact (1, 2). The potential increased generation of ROS by photoactivated nanomaterials, as well as being highly reactive, can more readily degrade planktonic algae and other important organics, thus influencing the productivity of an ecosystem (3). Increased ROS generation can also more efficiently render labile dissolved organic matter (DOM) into mineralized CO<sub>2</sub>. The increased recalcitrance of DOM may decrease microbial degradation rates of DOM and can potentially decrease the productivity of an ecosystem (4). Additionally, the increased concentration of ROS can potentially lead to increased interactions of radicals with the biota. Electron transfer quenching by nanomaterials is also an important reaction. During electron-hole pair recombination, some nanomaterials can scavenge an electron and thus decrease the natural ROS generation. The objective is to investigate the ability of nanosize metal oxides to increase or quench the generation of ROS in simulated freshwater and seawater systems. The ROS production and quenching kinetics will be investigated using a xenon arc lamp to irradiate a photoreactor.

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# Size effects of aqueous fullerene nanomaterials (FNMs) on degradation of organic compounds and bacterial activity

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Aqueous suspensions of some fullerene aggregates have been observed to produce reactive oxygen species (ROS) such as singlet oxygen and superoxide in the presence of ultraviolet (UV) or visible light [1-3]. In previous studies, we have shown that fullerol (hydroxylated fullerene) aggregates produce ROS via UV sensitization when measured by a variety of means: furfuryl alcohol degradation [3], electron paramagnetic resonance (EPR) [4], XTT reduction [4], and bacteriophage inactivation [5]. These properties suggest important medical and environmental engineering applications as well as possible toxic effects if released to the environment. While toxicity and reactivity of fullerenes has been studied over the last decade, very little consideration has been given to the impacts of their transformation on aquatic environments. In particular, we examine the effects of various sizes of fullerene nanomaterials (FNMs) on degradation of organic compounds and bacterial respiratory activity.

Suspensions of aqueous FNMs (i.e., fullerene, single wall (SW) and multi wall (MW) carbon nanotubes (CNT)) were prepared in deionized water (Nanopure, Barnstead, pH = 5.6) via sonication (Misonix 4000, Qsonica) for 5 hours. Then, each suspension was fractionated by successive filtration with membranes having nominal pore sizes of 1.2  $\mu\text{m}$ , 450, 100, 50, and 20 nm. Total carbon (TC) concentration of the fractionated suspension of aqueous FNMs was measured by total organic carbon (TOC) analyzer. Size distributions were measured by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Malvern Instrument, Bedford, MA). To assess bacterial toxicity of the fractionated FNMs, *Vibrio Fischeri* assay was performed as described by McConkey et al. [6]. Also to determine the effects of various sizes of fullerene nanomaterials (FNMs) on ROS production and degradation of organic compounds, batch tests were conducted under UV irradiation.

As shown in Figure 1, it was observed that TC concentration of fullerene ( $\text{C}_{60}$ ) was highest followed by SWCNT, and MWCNT in all size ranges. Then, these aqueous FNMs were applied to evaluate bacterial toxicity and photosensitized oxidation of probe organic compounds. As a result, as shown in Figure 2, colloidal suspensions of aqueous FNMs inhibited the activity of *V. fischeri*. Inhibition rates (% , left y-axis) were averaged on 58%, 33%, and 21% by fullerene, SWCNT, and MWCNT, respectively regardless of various sizes of the FNMs. However, normalized inhibition rates (% per TC concentration, right y-axis) increased as the size of FNMs decreased. Among three FNMs,

fullerene (C<sub>60</sub>) was consistently observed as the most effective microbial inhibitor followed by SWCNT and MWCNT. If the structural characteristics (size, surface area, etc) of aqueous FNMs are primary factors attributing to microbial activity then further investigation of ROS generation and oxidation of organic compounds by that will allow more understating of how these nanomaterials having different structures might impact the environment.

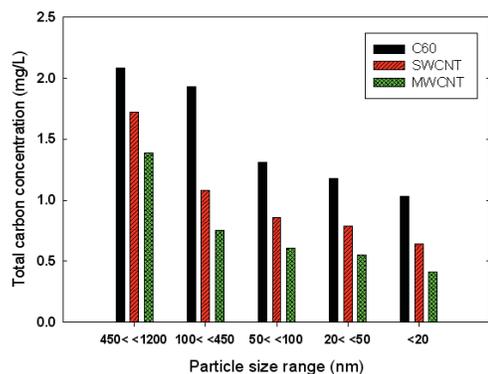


Figure. 1 Total carbon concentration of FNMs in different size ranges after 5 hours sonication

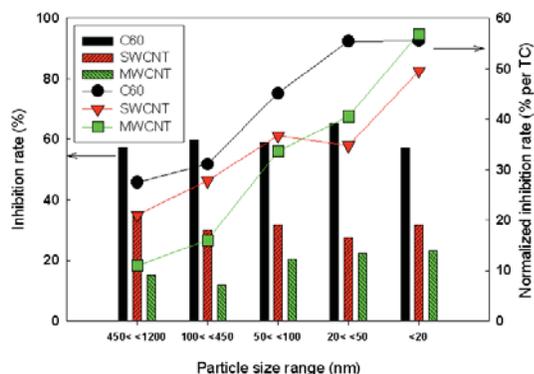


Figure. 2 Size dependent effect of the FNMs on respiratory activity of *V. fisheri* in water

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# **Container to Characterization: Impacts of Sonication, Nanoparticle Concentration and Ionic Strength on Metal Oxide Nanoparticle Stability**

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A study has been conducted to investigate the impact of experimental handling approaches on the state of the particles, from the container of dry particles to the characterization of suspended particles. Specifically, the effects of sonication, nanoparticle concentration, and ionic strength upon the size, electrophoretic mobility, and stability of model metal oxides (TiO<sub>2</sub>, CeO<sub>2</sub> and ZnO) were investigated. Titanium dioxide (TiO<sub>2</sub>), cerium dioxide (CeO<sub>2</sub>) and zinc oxide (ZnO) were selected as they are commonly used metal oxide nanomaterials in a wide array of applications including sunscreens, catalyst, gasoline additives, and polymer filters (1-5). Initially a sonication step is necessary to break up aggregated nanoparticles which occur when the dry metal oxide nanoparticles are first suspended in water. An approach to prepare reproducible and well dispersed nanoparticles suspensions was determined over the range of solution chemistry tested. Additionally, the nanoparticle suspension required a subsequent sonication step following dilution of the stock solution for use in experimentation. Next, the variation in nanoparticle concentration was observed to cause a change in solution pH. This shift, caused by the metal oxide nanoparticles not only resulted in a shift in pH, but also in measured particle electrophoretic mobility. Finally, ionic strength was found to affect the stability of these metal oxide nanoparticles, with enhanced aggregation with increasing ionic strength. The detailed analyses of the three metal oxide nanoparticles will be presented, along with recommendations for particle handling to ensure reproducible, consistent particles.

## **Acknowledgements:**

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# Effects of Composition on Structural Aspects of Naturally Occurring Ferrihydrite

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Ferrihydrite is a poorly crystalline iron oxide nanomineral (2-7 nm) found in a variety of natural surface environments. As a result of its high surface area (up to 600 m<sup>2</sup>/g) and its chemical reactivity, it is an important environmental sorbent, and plays an essential role in the geochemical cycling of pollutant metal(loid)s. Despite its environmental relevance, this mineral is one of the least understood environmental solids in terms of structure (bulk and surface), compositional variations, and the factors affecting its reactivity. Under natural aqueous conditions, ferrihydrite precipitates in the presence of several inorganic species such as aluminum, silica, phosphate, etc., or in the presence of organic matter. These impurities can affect the domain size, composition, and molecular-level structure of ferrihydrite, thus modifying fundamental properties that are directly correlated with solid-phase stability and surface reactivity. In this study we have characterized a suite of ferrihydrite samples of variable Al, Si, and C content by synchrotron-based techniques (high energy x-ray total scattering and pair distribution function (PDF) analysis and scanning transmission x-ray microscopy) in order to place constraints on their short- and intermediate-range structure, composition, and association with organic matter. Despite the significant concentrations and variations in total Al (4.8–15.1 wt % Al<sub>2</sub>O<sub>3</sub>) and Si (2.6–9.1 wt % SiO<sub>2</sub>) and the presence of significant organic carbon (0.3 wt %) in the samples studied, the PDFs of natural ferrihydrites indicate that their structure is fairly comparable to pure, synthetic ferrihydrite (1). However, with increasing impurity content, there are several observable differences in the PDFs, including a decrease in the coherent scattering domain length, and a reduction of the Fe-Fe corner-sharing correlations. We attribute these changes primarily to the presence of strong binding ligands such as silica and organic matter, both of which are known to affect Fe polymerization and poison particle growth. It is likely that the presence of these ligands at the ferrihydrite surfaces will alter significantly their surface composition and reactivity in the environment.

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# Effects of silver nanoparticles on microbial activity in sediment and streamwater

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Silver has long been known to have antimicrobial properties, and silver nanoparticles have been shown in lab studies to have antimicrobial properties at lower doses than ionic forms of silver [1]. In an attempt to harness its antimicrobial properties, nanoparticles of silver are becoming increasingly common in consumer goods ranging from clothing and children's toys to supplements and cosmetics. Through the use, aging, and cleaning of these products, silver nanoparticles can enter the environment directly, or through sewage treatment facilities. Given that microorganisms are essential to decomposition and nutrient cycling, and that nanosilver is known to be antimicrobial, we pose the question: what are the effects of nanosilver on microbes in natural ecosystems? To answer this question, we have added silver nanoparticles to streamwater and sediments and examined several indices of microbial activity including microbial respiration, microbial biomass, and enzyme activity.

Our work suggests that the impact of Nanoamor PVP coated silver nanoparticles (10nm) depends on the environment to which they are added. In additions to stream sediments of up to 250 mg Ag/L, silver nanoparticles did not cause decreases in either microbial biomass or respiration. In streamwater, however, Ag nanoparticles added at 7.5 and 75mg Ag/L caused a decrease in microbial respiration and microbial biomass. In streamwater, the magnitude of the effects of nanosilver correlated with the ionic silver concentration in the incubations.

While the sensitivity of microbial biomass and respiration to silver nanoparticles in sediment and streamwater differed, in both environments silver nanoparticles caused an increase in phosphatase enzyme activity. Phosphorous is often limiting in stream ecosystems, and while P concentrations did not increase from 2  $\mu\text{g/L}$  in streamwater when isolated from sediment (averaging 2  $\mu\text{g/L}$  across nanosilver additions), phosphate concentrations rose from 120  $\mu\text{g/L}$  to 270  $\mu\text{g/L}$  in sediment slurries with addition of 250mg/L Ag nanoparticles. Thus increased phosphorous concentrations in sediments could have ecosystem level effects if they increase phosphorous availability to algae and vascular plants.

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# Nanophytotoxicity assessment and potential biotransformation of ZnO in selected desert plants

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Nanotoxicological studies in plants have been mainly focused on crops and model plants (1, 2). Given that arid and semiarid areas account for about 30-40% of total land in the world (3), a nanophytotoxicity survey should also include desert plants. In this work, we studied the effect of ZnO nanoparticles (NPs) on seed germination and plant growth on the desert plants mesquite, tumbleweed and palo verde. X-ray absorption spectroscopy (XAS) was used to determine the potential ZnO NP biotransformation. Plant seeds were germinated in 0, 500, 1000, 2000, and 4000 mg L<sup>-1</sup> ZnO NPs. Results indicated that none of the NP concentration significantly affected ( $P < 0.05$ ) the germination of the species tested. The length of tumbleweed root seedlings was not affected; however, a root length reduction of 16 % was observed in palo verde at the NP concentration of 4000 mg L<sup>-1</sup>. In mesquite, levels of 2000 and 4000 mg ZnO L<sup>-1</sup> caused root length reductions of 65 and 64 %, respectively, which suggests that the IC<sub>50</sub> was between 1000 and 2000 mg L<sup>-1</sup>. Zn concentrations in plant tissues were of 88, 820, and 1600 mg kg<sup>-1</sup> for palo verde, mesquite, and tumbleweed, respectively. The XAS analysis indicated that Zn within tissues was present as Zn(II) coordinated to oxygen.

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## **Influence of Silver Nanoparticles on *Drosophila* Development and Behavior**

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Developing early third instar larvae from the wild type (Canton S strain) *Drosophila melanogaster* were exposed to various concentrations of Ag- nanoparticles through feeding. Unexposed flies of the same genotype were used as controls. Two measurement parameters used were pupal formation and adult eclosion. In order to see how exposure to the nanoparticles can influence their neuromuscular function, we also measured the vertical climbing behavior of the adult flies exposed to Ag- nanoparticles.

Silver citrate stabilized nanoparticles, at known levels up to 0.001M expressed as Ag, were synthesized by microwave thermolysis and maintained in an aqueous medium at pH 7.0. From the nanoparticle stock solution containing particles of 45.8 nm, doped sucrose media was prepared and experimentally verified by resonance light scattering as physically and chemically stable. Nanoparticles induced in 1% sucrose, the most optimum feeding medium for *Drosophila*, maintained a stable particle size of 71.9 nm and exhibited a constant plasmon resonance over a 14 day period, which exceeds the duration of the feeding experiments. Preparation of cation-free silver nanoparticle and silver cation containing media allowed comparative scrutiny of the impact on *Drosophila*.

Following exposure to Ag-nanoparticles at concentrations  $9 \times 10^{-6}$  M and 0.0009 M in 1% sucrose, pupal formation was unaffected. However, exposure to silver nitrate turn out to be very potent. 0.01M Silver Nitrate caused <1% pupal formation and 100% adult lethality. We therefore fixed several sublethal concentrations of silver nitrate ( $10^{-6}$ — $10^{-3}$  M) to determine its effect on pupal formation and adult eclosion and found a nice dose-dependent relationship between Silver Nitrate concentration with pupal formation and adult eclosion rate. The highest sub-lethal concentrations of silver nitrate ( $10^{-3}$  M) showed the most significant effect on pupal formation as well as on adult eclosion.

As opposed to silver nitrate, exposure to silver nanoparticles (concentrations used 1-3%) was inconsequential in terms of pupal formation, adult eclosion, and locomotor ability. We are now trying to deliver the silver nanoparticles at a higher concentration and by changing the delivery route by microinjection of silver nanoparticles into developing embryos.

# Effects of Metal Oxide Nanomaterials on Sea Urchin Embryo Development

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Little is known regarding the effects of nanomaterials (NMs) on the development of marine organisms. We have focused on the impacts of metal oxide NMs on the development of embryos from the white sea urchin (*Lytechinus pictus*). Three metal oxide NMs (ZnO, CeO<sub>2</sub>, and TiO<sub>2</sub>) were prepared as stock solutions in filtered sea water with or without 10 ppm alginate, and then diluted into 12-well culture plates for embryo exposure. Newly fertilized sea urchin embryos were exposed to NMs for approximately 96 hours, until the pluteus stage, and then assessed for normal development. ZnO was toxic to developing sea urchins as low as 1-10 ppb, with greater than 50% of embryos showing abnormalities at 100ppb. Using alginate as a source of dissolved organic carbon (DOC), we found that DOC increased the toxicity of ZnO. Two other metal oxide NMs, TiO<sub>2</sub> and CeO<sub>2</sub>, had no effect on development up to 50 ppm, and alginate did not increase their potency. The effects of ZnO NM on development did not appear to be stage-specific but rather, the effects were a result of exposure duration. Finally, using ZnO NMs that were covalently conjugated with FITC, we found that FITC-ZnO NM was incorporated into cells of the embryos. Using scanning laser confocal microscopy, we found ZnO-FITC fluorescence throughout the embryo, with a few select cells showing a much greater accumulation of FITC-ZnO. FITC-ZnO was excluded from the nuclei of cells.

## **Evaluating Hydrophobicity of Nanoparticles**

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Nanoparticles are increasingly used for a variety of purposes. Recent publications report their threatening to cells as well as living creatures. The hydrophobicity is an important property of nanoparticles which significantly affects their transport and fate in the environment. Currently, however, there is barely any effective way to characterize such a property down to the nano scales. We show here a novel technique developed to quantify such a property. It is based on monitoring the evaporation of water molecules with and without the presence of nanoparticles. Compared to traditional method, this method is easy, accurate, and robust. The grading of hydrophobicity of the various fine particles is therefore feasible, which is important to quantify the nanotoxicity with respect to particle properties.

# Surface Functionalization of Nanoparticles to Produce a Bio-Interactive Material

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Modification of mesoporous silica through condensation of organosilanes onto and into mesoporous silica nanoparticles has imbued this material with a wide variety of different properties. By attaching different organic substituents to the surface of the nanoparticles, the bio-particle interactions can be altered to change the bio-availability and toxicity to cells. Condensation of linkers such as amines or isothiocyanate enables attachment of various organic groups that provide novel function to the mesoporous silica platform, including containment of molecules and release from the mesoporous framework. Surface modification also provides reactive locations to covalently attach biomolecules such as proteins or peptides. These biomolecules allows for cell specific uptake of the nanoparticles based on the expression of receptors for the specific protein or peptide. In addition to surface functionalization, co-condensation can be utilized to modify the particles and make them fluorescent for monitoring of the particle location within cells using confocal microscopy. These types of modifications have important consequences on cellular function and response, animal biodistribution and dispersal into the environment.

# **Ecotoxicity of nanoparticulate metal oxides on soil microbial community composition and function**

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Engineered nanoparticles (ENPs) show antibacterial activity in pure culture conditions, which imply that ENPs may be environmental toxins. However, little is known about the effects of ENPs on complex microbial communities and processes in terrestrial ecosystems. Nitrogen (N) mineralization and nitrification are two important processes in the soil N cycle. These processes supply most of the N used by terrestrial plants, and nitrification is critical in regulating N<sub>2</sub>O and NO fluxes to the atmosphere. This study aims to quantify the effects of two widely-used nanoparticulate metal oxides (TiO<sub>2</sub> and ZnO) on soil N mineralization and nitrification by incubating microcosms (for 15, 30, 45 and 60 days) with different concentrations of TiO<sub>2</sub> (0, 500, 1000 and 2000 μg g<sup>-1</sup> fresh soil) and ZnO (50, 100 and 500 μg g<sup>-1</sup> fresh soil). We measure net mineralization and nitrification, and use <sup>15</sup>N dilution to measure gross NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> turnover rates. Total respiration by CO<sub>2</sub> production is measured from the headspace of the sealed bottles. We measure dehydrogenase, protease and chitinase activities for assessing overall community metabolism, and intra- and extracellular N depolymerization, respectively. Overall microbial community and nitrifier composition and diversity are examined by PCR T-RFLP, while their biomass is measured by substrate induced respiration (SIR), the amount of total extracted DNA, and real-time PCR using group-specific primers. The bioavailability of ENPs is assessed by extracting the soil solution then analyzing it by ICP-MS. This study is advancing our understanding of the environmental and ecological risks of ENPs in terrestrial ecosystems.

## High throughput multiparametric cytotoxicity screening for predictive toxicological assessment of nanomaterials

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Towards the realization of a verifiably safe nanotechnology, we report a high throughput toxicity screening based on hierarchical oxidative stress paradigms and materials re-design to ameliorate the hazardous characteristics of zinc oxide (ZnO) nanoparticles. The hazard assessment of ten different nanoparticles at multiple doses and durations in bronchial epithelial and macrophage cell lines were carried out by employing a combination of fluorescent dyes that can be loaded contemporaneously. An integrated series of cytotoxic effects were quantified by automated epifluorescence microscopy. Nanoparticles exhibited differential toxicity pattern as evaluated by cytotoxic effects such as mitochondrial superoxide generation, mitochondrial depolarization, increased intracellular calcium flux, and surface membrane leakage. As an example of purposeful amelioration of toxicity based on mechanistic understanding, we report the reduction in toxicity of ZnO nanoparticles achieved by doping with iron. Doping of ZnO with incremental concentrations of iron reduced the dissolution of Zn<sup>2+</sup> ions, consequently reducing the toxicity in a concentration dependent manner. Hence, by combining *in vitro* detection of a hazardous nanomaterial property that can be correlated to *in vivo* toxicity and safe material design, this work exemplifies a rational approach towards safer nanotechnology applications.

# ZnO Nanoparticle Toxicity on Mesquite (*Prosopis* sp.) Sprouts

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The rapid development of nanotechnology has created an urge for the assessment of possible health and environmental hazards caused by metal nanoparticles (NPs) (1)(2). As the first trophic level, plants should be tested in order to determine their response and possible role in the fate and transport of NPs. Seed germination has been used to evaluate the toxicity of metals and metalloids at the early growth stage of plants (3). In this study, mesquite (*Prosopis* sp.) seeds were germinated in ZnO NPs suspensions (0, 50, 100, 200, 400, 800, and 1600 mg L<sup>-1</sup>) and Zn<sup>+2</sup> solutions (0, 0.05, 0.5, 5, 10, 50, and 250 mg L<sup>-1</sup>). Percent germination, and the root and shoot elongation were measured to determine the toxicity of the ZnO NPs. Seedlings were digested and Zn was quantified using inductively coupled plasma-optical emission spectroscopy (ICP-OES). Results showed that none of the treatments significantly decreased seed germination or shoot elongation in mesquite plants. At 1600 mg L<sup>-1</sup> of ZnO and 250 mg L<sup>-1</sup> of Zn<sup>+2</sup> the seedlings accumulated 1000 and 470 mg kg<sup>-1</sup> and the root size decreased by about 30 and 10%, respectively. These data suggest that the treatments used in this research were not significantly toxic for mesquite, indicating that this desert plant may display some tolerance to ZnO NPS.

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# Managing the Uncertainty from Nanomaterials in Municipal Solid Waste

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As nanotechnology evolves and nanoparticles become more prevalent in today's market a growing amount of research explore the potential hazards of nanotechnology on human health and the environment. Some research focuses on how current EPA regulations can apply or be updated to more effectively apply to nanomaterials. Waste containing nanomaterials (Figure 1) can occur at different stages in the lifecycle of nanomaterial products and various regulations apply to those products and resulting nanomaterial waste. Even though nanotechnology is never specifically mentioned in EPA regulations, the EPA has the authority, if necessary, to manage nanomaterials through TSCA and FIFRA and manage nanomaterial waste through regulation such as the CAA, CWA, and more notably solid waste through RCRA (1). It is unclear whether current standards and

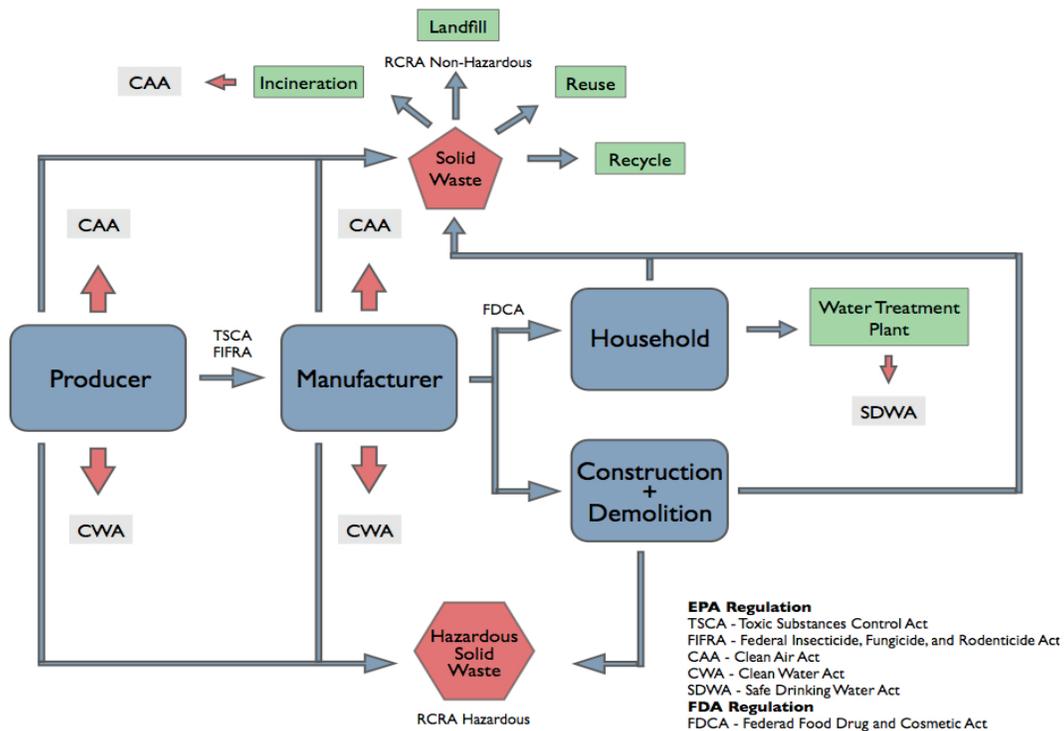


Figure 1. Potential waste streams containing nanomaterials and the Agency/Act under which those wastes can be subject to regulations.

regulations are sufficient to manage potentially hazardous nanomaterials, but probable gaps in applying RCRA to nanomaterial waste have been previously identified (2,3). These gaps have applied to entities producing nanomaterials or manufacturing with nanomaterials on the commercial level and have focused on the process of classifying waste as hazardous (thus subjecting it to more stringent regulation). Household waste however, is exempt from RCRA hazardous waste classification. Consumer goods are subject to little environmental regulation; while guidelines exist to ensure safety of consumer products, most of these guidelines are imposed to protect consumer health rather than to protect the environment. Few, if any, reports have explored how to contain and control nanoparticles once they are in the hands of the consumer.

As nanoparticles continue to be integrated into consumer goods at an increasing rate, it is important to assure that household waste does not negatively impact environmental health regardless of its disposal technique. Household waste will likely be the largest (non-point) source of nanomaterials to the environment and their disposal is currently unregulated. Fate and transport of nanoparticles in the primary methods of household waste disposal should be assessed to ensure that they are not unintentionally released. In the USA, the majority of household solid waste is sent to landfills where leaching or transformation of nanoparticles may occur. Recycling and incineration additionally pose the threats of airborne nanoparticles that can be harmful to human health.

In our research we aim to identify how common disposal techniques of household waste can potentially release nanoparticles into the environment. Given these probable exposure pathways, we suggest measures to minimize the risk of household products containing nanoparticles.

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# **In vitro toxicity testing of nanoparticles deposited at the air-liquid interface**

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A major difficulty in assessing the inhalation risk of aerosolized nanoparticles is that conventional in vitro toxicity testing requires the preparation of suspensions of particles in culture medium. The process of suspending particles in medium can cause extensive aggregation and modification of surface characteristics, resulting in particles significantly altered from those expected to deposit in the human lung upon inhalation (1). An alternative approach is to deposit aerosol particles directly on cells at the air-liquid interface (ALI), thus avoiding the artifacts associated with creating particle suspensions and providing an in vitro exposure that is relevant for measuring effects from inhaling nanoparticles (2). The objective of this work is to measure the dose in terms of particle number and size at the ALI and compare the sensitivity of the ALI exposure to the conventional exposure with particle suspensions in submerged cultures. Results from past work with soot particles from a diesel engine and methane flame will be used to guide new experiments involving engineered nanoparticles (3).

Human lung cells were grown in Transwells (Corning), which leaves the cell surface exposed to air allowing for direct contact with aerosolized particles. This ALI arrangement was used to expose cells to particles in diesel exhaust and a methane flame with diameters of 110 nm at a concentration of approximately  $10^5$  #/cm<sup>3</sup>. The ALI exposure was compared to conventional in vitro exposure, where particles are collected on filters and suspended in cell culture medium. The toxic and inflammatory responses of the two exposure methods were compared.

The number of particles that deposit at the ALI was approximately  $10^6$  #/cm<sup>2</sup>, which is about the same as that expected to deposit in the tracheobronchial region of a human lung. However, the size of the particles depositing on the ALI were skewed towards larger diameters (~300 nm) as the gravitational deposition mechanism is more efficient at the larger sizes, unlike that expected to deposit in the lung (~80 nm).

The toxicity (measured as a reduction in viability) and the inflammatory response (measured as an increase of inflammatory marker IL-8) for cells exposed to diesel and flame particles is shown in Table 1. For both particle types, similar responses of viability and inflammation were observed. However, the comparable response of ALI exposed cells was observed at doses one and two orders of magnitude lower than for cells exposed to particle suspensions, for the diesel and flame particles respectively.

Table 1. Viability and inflammatory response of cells exposed to diesel and flame particles at the ALI and in suspension.

Exposure Method	Dose ( $\mu\text{g}/\text{cm}^2$ )	Diesel Particles		Dose ( $\mu\text{g}/\text{cm}^2$ )	Flame Particles	
		Viability (% Control)	IL-8 (% Control)		Viability (% Control)	IL-8 (% Control)
ALI	0.09	96.4 $\pm$ 4.9	136 $\pm$ 25	0.04	115 $\pm$ 34	169 $\pm$ 22*
Suspension	1.88	94.4 $\pm$ 2.4	148 $\pm$ 18*	4.83	70.5 $\pm$ 4.8*	149 $\pm$ 39*

\*Statistically significant at  $p < 0.01$  compared to control.

We suspect that the ALI exposure method is more sensitive because the inflammatory effects of particles in suspension are reduced by the surfactant coating and aggregation that occurs in the culture medium. The diesel and flame particles rapidly formed aggregates that were approximately the same size as the cells and about 10 times the size of particles depositing on the ALI.

The ALI exposure method has been shown to be an in vitro approach that is relevant for an inhalation route and is more responsive than the conventional exposure to particle suspensions. In future experiments we plan to enhance the deposition of smaller particles by generating an electric field and relying on the electrophoretic force to drive charged particles to the cell surface (4). With this design, lung cells can be exposed to substantial numbers of aerosolized engineered nanoparticles, such as silver and metal oxides, as single particles rather than large agglomerates.

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# Precipitation of mercury and zinc sulfide nanoparticles in the presence of natural organic matter

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In anaerobic aquatic systems, metal sulfides can control the mobility and bioavailability of trace metal pollutants such as zinc and mercury. Nanoparticulate metal sulfides in particular may play a significant role in regulating dissolved metal concentrations and the availability of trace metals for biological uptake. However, the processes responsible for the persistence of metal sulfide nanoparticles in the aquatic environment are not well understood. The objective of this study was to explore the potential for HgS and ZnS nanoparticles that are coated with or co-precipitated with natural organic matter (NOM). HgS and ZnS precipitation experiments were conducted in the presence of organic acids such as humic substances and amino acids that are prevalent in surface water and sediment porewater. Dynamic light scattering was used to monitor the size of metal sulfide colloids precipitating over time. Nanoparticle growth rates were monitored as a function of monovalent electrolyte and NOM type and concentration. The results indicated that thiol-containing organic acids such as cysteine and thioglycolate decreased the rate of growth of HgS and ZnS particles whereas the hydroxyl-containing analogues (serine and glycolate) did not affect growth rates. Humic substances also decreased observed growth rates of HgS and stabilized colloidal aggregates smaller than 0.2  $\mu\text{m}$  for at least 8 hours. HgS and ZnS particles that formed in the presence of humic substances and thiolates were able to pass through conventional filters (0.2  $\mu\text{m}$ ) and appeared to consist of aggregates of nanocrystals in TEM images. The interaction between the metal sulfide nanoparticles and humic substances was investigated in further detail by comparing nanoparticle growth and aggregation rates with NOM fractions isolated from several different freshwater systems. Nanoparticle stabilization by NOM appeared to be positively correlated with NOM aromatic content ( $r^2=0.46$ ) and specific UV absorbance at  $\lambda=280\text{nm}$  ( $r^2=0.66$ ), and weakly correlated with reduced sulfur content of the NOM ( $r^2=0.08$ ). Humic acid fractions appeared to reduce particle growth rates to a greater extent than fulvic acid fractions, possibly due to compositional differences such as higher aromatic and reduced sulfur content in the humic acids. This study suggests that HgS and ZnS nanoparticles could exist in surface waters and porewater of contaminated sediments as a result of mineralization reactions which are kinetically-hindered due to specific surface interactions with thiol-containing organics or hydrophobic interactions with aromatic functional groups in humic substances. These interactions may be contributing factors in the persistence of naturally occurring metal sulfide nanoparticles in the aquatic environment. Further studies are needed to address the importance of nanoscale metal sulfide particles for governing the reactivity and bioavailability of trace metals in the environment.



# **Synthesis and Characterization of Mackinawite (FeS) Nanoparticles for Use in Oxyanion Sorption Experiments**

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In our continuing quest to understand the implications of nanomaterials in the environment, we synthesize nanominerals that also have potential commercial nanotechnology implications. Iron sulfides, one such group of materials, are common phases in reduced environments, and yet there is much to be understood about their importance in environmental and geological systems. A good example in the iron sulfide system is mackinawite (FeS), a metastable precursor to pyrite (FeS<sub>2</sub>; the most common sulfide in the Earth's crust) that forms in anoxic, sulfidic sediments (1) such as acid mine drainage systems and various coastal environments. As with iron (oxy)hydroxides, it has been shown to react with contaminants such as cationic heavy metals as well as oxyanions. Given that nano-sized mineral phases are often dramatically more reactive than bulk phases due to their increased surface area to volume ratios and also due to potentially more reactive surfaces on a surface area normalized basis, contaminants will have a greater tendency to adsorb onto, or be incorporated into, these particles and be transported through environments in different ways than we might expect (2). Therefore, it is important that we study these nano-sized phases in relationship to trace metal cycling in contaminated environments.

To date only a few studies have been performed regarding the sorption onto mackinawite of trace metals and metalloids including arsenic, antimony, selenium, chromium, and uranium. Very few of these use well-characterized nano-sized mackinawite for their sorption experiments. Our aims are to 1) synthesize nano-sized mackinawite of distinct size distributions, 2) understand the state of the particles (size and shape, fresh vs. aged) and its impact on reactivity, and 3) perform batch sorption experiments involving trace elements like inorganic arsenic to create sorption isotherms. To do this, we are employing a suite of instrumental techniques, including: Transmission Electron Microscopy (TEM), X-ray Diffractometry (XRD), Dynamic Light Scattering (DLS), and BET surface area measurements.

Preliminary work on the mackinawite synthesis has shown that smaller particles form at low pH, and that they have a strong tendency to aggregate, as observed with the TEM. XRD and electron diffraction patterns from the TEM confirmed the formation of mackinawite particles. DLS showed that after aging for only 1 day, the size distribution by intensity peaked around 100 nm and 4000 nm in buffered solution, in contrast to fresh samples that had peaks around 10 nm and just under 100 nm. This is consistent with work done by Michel et al (3), who reported unpublished DLS results showing strong tendencies for FeS to aggregate in solution and also during filtering and drying.

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# Organic surface coatings affect fate and potential for exposure to Fe<sup>0</sup> and Fe-oxide nanoparticles in heterogeneous porous media

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Zerovalent iron nanoparticles (nZVI) are attractive for in situ remediation of contaminated groundwater due to their high reactivity with chlorinated organic compounds and mobility in the porous media. Polyelectrolyte surface coatings are used to inhibit nZVI aggregation and enhance the transport of them in the subsurface. Polyelectrolyte surface coatings alter the nZVI surface chemistry which controls the mobility, fate, interaction with aquifer biota, and potential toxicity. The ultimate fate of surface modified nZVI depends on the stability of the coatings against desorption, biodegradation and the oxidation of Fe<sup>0</sup>. In addition, physical and chemical heterogeneity such as groundwater pH and the presence of fines and clays may decrease the mobility of anionic polyelectrolyte coated NZVI due to enhanced aggregation and deposition. This study measured the rate and extent of desorption of polyelectrolyte coatings used to stabilize nZVI, including polyaspartate, carboxymethyl cellulose, and polystyrene sulfonate. Also this study determined the effect of pH, the presence of fines and clay particles on the mobility of surface modified nZVIs.

Desorption of polyelectrolyte was slow, with less than 30 wt % of each polyelectrolyte desorbed after 4 months. The higher molecular weight polyelectrolyte had a greater adsorbed mass and a slower desorption rate for PAP and CMC. nZVI mobility in sand columns (Figure 1) after 8 month of desorption was similar to freshly modified nZVI, and significantly greater than unmodified nZVI aged for the same time under identical conditions. Based on these results, polyelectrolyte modified nanoparticles will remain more mobile than their unmodified counterparts even after aging. Polyelectrolyte modified nanoiron showed a limited mobility especially below pH 7, and the presence of 2 % (w/w) clay particles caused more deposition (filtration) of nZVI in saturated sand columns (Figure 2). Decreased mobility was partly caused by nZVI aggregation resulting from decreased charge stabilization at low pH, and partly due to the enhanced deposition onto the clay particles which exhibit pH dependent charge. Transport of polyaspartate (2.5 kg/mol and a weak polyelectrolyte)-modified NZVI was significantly more pH-sensitive than for a triblock copolymer (MW=105 kg/mol and a strong polyelectrolyte)-modified NZVI. These results indicate that differences in coating types and subsurface hydrogeochemical conditions affect the transport of nZVI and Fe-oxide nanoparticles and must be considered carefully when designing site-specific

remediation strategies Because the coatings do not readily desorb and show long term mobility, the potential for surface modified nZVI toxicity will remain as that of coated nZVI, unless soil bacteria can degrade the adsorbed polyelectrolyte coatings. Transport of surface coated nZVI in real aquifers with clay particles and a large particle size distribution at pH<7 will be limited after the emplacement.

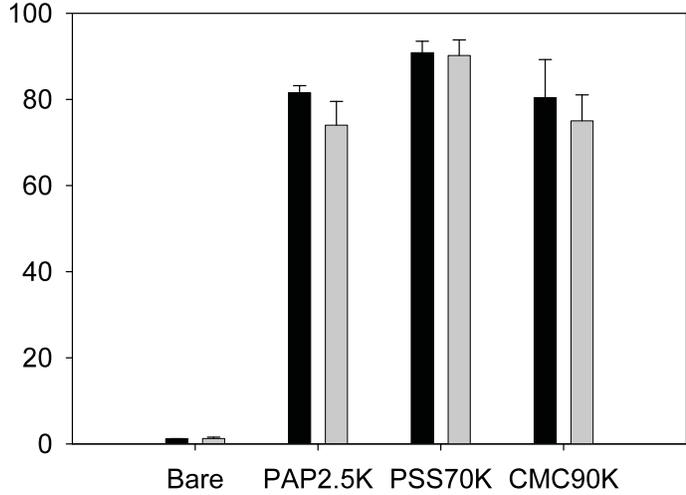


FIGURE 1. Percent mass of RNIP eluted through a 12.5-cm saturated silica sand column in 1mM NaHCO<sub>3</sub> for freshly modified particles with excess polymer (black bars) and after 8 months for aging and desorption (gray bars). Porosity is 0.33, pore water velocity is 1.08 x 10<sup>-3</sup> m/s, and particle concentration is 1 g/L.

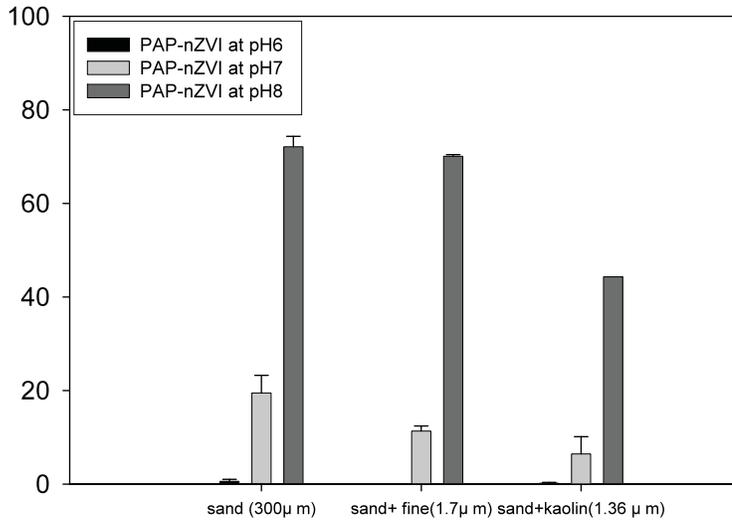


FIGURE 2. Percent mass of PAP2.5K modified nZVI with initial particle concentration with 300 mg/L eluted through 15 cm silica sand, sand+ 2 % (w/w) silica fines (1.7 µm) and sand + 2 % (w/w) clay (1.36µm) columns at pH=6, pH=7 and pH=8 with porosity of 0.33. The linear pore water velocity was 3.2 x 10<sup>-4</sup> m/s and ionic strength was controlled at 10 mM NaCl + 1 mM NaHCO<sub>3</sub>.

# Modeling effects of soluble cadmium salts and CdSe quantum dots on *Pseudomonas aeruginosa*

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Nanoparticles exhibit fundamentally different behavior than larger particles, even when made of the same materials. Understanding how this difference affects ecological processes is a necessary step in responsible utilization of nanoparticles, especially when materials contained within are known to be toxic. Bacteria provide a good starting point for the research. Experiments by Priester et al. (2009) demonstrate that the growth rate of *Pseudomonas aeruginosa* declines more rapidly in response to Cd(II) from CdSe quantum dots (QDs) than from soluble salts. Any experimental approach has, however, limited ability to distinguish which metabolic processes are most strongly affected. By describing acquisition and utilization of energy, bioaccumulation of toxicants, and toxic effects on physiological processes, Dynamic Energy Budget (DEB) theory provides tools to investigate further. Using this approach, we identify metabolic processes that are affected by the Cd(II) ions in both cases. Differences in cross-membrane methods of transport could be responsible for the observed differences in responses to exposure. We explore the hypothesis that the weaker response to soluble Cd salts treatment is caused by saturation of Cd(II) ion cross-membrane transport mechanisms, whereas the stronger response to QD treatment is due to ability of QDs to enter the cell and deliver Cd(II) ions directly.

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# Size-dependent Structure and Reactivity of Silver Nanoparticles under Different Environmental Conditions

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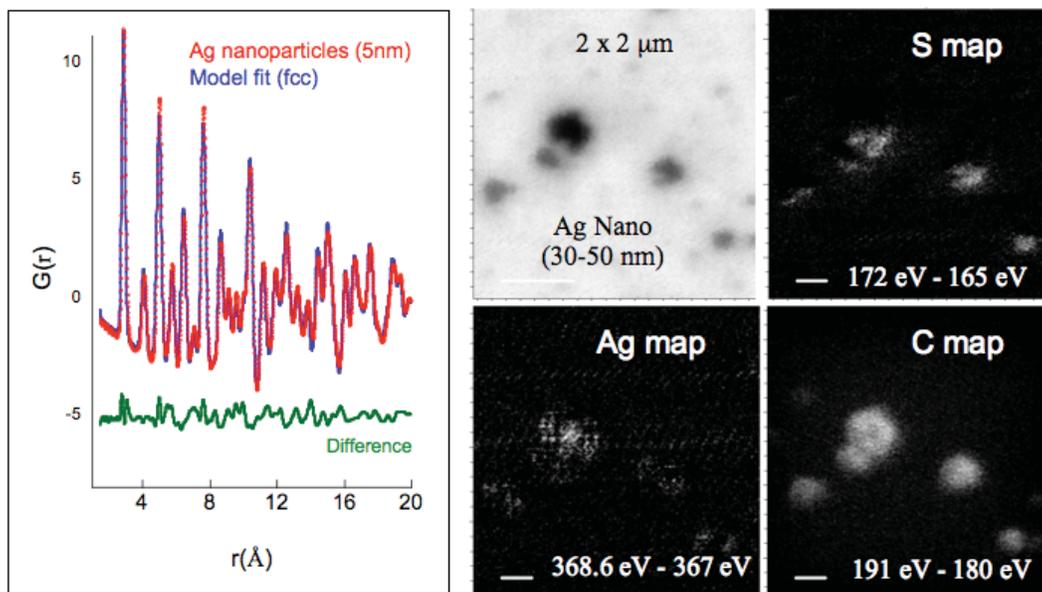
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Rapid development of nanotechnologies has raised environmental questions concerning the impact of nanoparticles on microbes and other organisms. Ag nanoparticles are of particular interest because they are the most widely used in the nanotechnology industry and are expected to be released into the environment in relatively large amounts. In this context, the focus of our study is on the structure and reactivity of near-spherical Ag nanoparticles as a function of particle size (5-100 nm) and environmental conditions (*e.g.*, Eh, pH, solution composition). The first step consists of synthesizing silver nanoparticles using a modification of the polyol process [1]. To prevent aggregation, the nanoparticles are coated with polyvinyl pyrrolidone (PVP), which is strongly adsorbed but can be decomposed by heating [2]. Zeta potential measurements combined with size measurements show that the nanoparticles are stable between pH 3.3 and 11 with a strong negative surface (-30 to -60 mV). Total scattering experiments combined with pair distribution function analysis revealed that silver nanoparticles are crystalline with a fcc lattice for 5 to 50 nm particle sizes (see Figure 1, left). We have observed no changes of the lattice parameter ( $a = 4.09 \pm 0.01 \text{ \AA}$ ) with increasing particle size.

A focus of our current study is characterization of surface products on Ag nanoparticles formed under different conditions that will impact the reactivity of the nanoparticles. Preliminary surface characterization studies have been performed, including Scanning Transmission X-ray Microscopy (STXM) and X-ray Photoelectron Spectroscopy (XPS). Both techniques confirm the presence of PVP adsorbed at the nanoparticle surfaces. These two techniques appear to be well adapted for characterizing the polymer stability under different conditions as well as the silver nanoparticle surface corrosion products. Indeed, STXM results reveal the presence of sulfur at the surfaces of the silver nanoparticles exposed to ambient air during the past few months (Figure 1, right). An interesting result is that PVP does not prevent the sulfidation of the silver nanoparticle surfaces. Sulfidation of silver nanoparticle surfaces results in the formation of acanthite ( $\text{Ag}_2\text{S}$ ), which is a well-known process [3]. However, the effect of particle size on sulfidation rate is unknown and needs to be further explored. The formation of other surface products will be studied such as the precipitation of AgCl at the nanoparticle surfaces in NaCl solutions. The stability of PVP will be also investigated under different

conditions (*i.e.* pH, Eh, solution chemical composition). The chemical reactivity of our silver nanoparticles will be investigated as a function of particle size using an approach similar to those in our past studies of Zn sorption products on hematite nanoparticles and microparticles [4]. Zn(II) will be used as a probe ion to investigate the reactive sites of the Ag nanoparticle surfaces. Zn(II) sorption on Ag nanoparticle surfaces prepared under different environmental conditions will be investigated by measuring pH-dependent and concentration-dependent Zn adsorption isotherms. Sorption products will be characterized using Zn K-edge EXAFS and TEM.



**Figure 1.** (Left) Pair Distribution Function (PDF) of 5 nm silver nanoparticles. The difference between the experimental data and the refined fit of the model is shown below. (Right) STXM maps of S, C, and Ag for 30-50 nm silver nanoparticles. The white scale bar in the lower left corner of these maps is 200 nm in length.

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# Unsupervised Feature Subset Selection with Feature Similarity for the Analysis of Chemical and Nanoparticle Toxicity

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Developing data driven models, such as quantitative structure-property relations (QSPR) and environmental multimedia distribution of nanoparticles, is an effective approach to assessing the potential impact of nanomaterials on environment and human health. With recent innovations in nanotechnology, including experimental nanoparticle characterization methods, not only the size of the data on the physicochemical properties of nanoparticles but also their dimension has been rapidly accumulating. In most cases, not all of the attributes are related to the data modeling (mining) tasks. The presence of insignificant and unrelated attributes will inevitably complicate data analyses and worsen the modeling result, especially for mining high dimensional data. In order to address the above challenge, the use of an unsupervised feature selection method is proposed. This method aimed to maximize the similarity between the selected and unselected features. With the feature similarity defined with respect to their neighbors, the features which are similar to the unselected ones but dissimilar to those selected are kept. As a result, the unselected features can be well represented by the selected ones because of the high similarity between them, and they can then be removed to eliminate redundancy. The superiority of this method, in terms of efficiency and performance, was evaluated extensively over supervised and unsupervised applications to various real-life data sets including a chemical data set large in both size and dimension.

**Key words:** Feature Selection, Feature Similarity, Unsupervised Learning, Dimensionality Reduction,

## **Nano: the hot new thing... that has been around for a while.**

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In human history, there has been the stone age, the bronze age, the iron age, and now we are living in what even the very serious US-DOE calls the nano age. The discovery of the C<sub>60</sub> molecule in 1985 marks the "historic" beginning of this era. It took then about a decade before nanotechnologies got out of the research laboratories and slowly initiated the boost that we are still witnessing today; public awareness of nanomaterials (and associated concerns) followed just a few years later. This is the typical timeline described in a number of print and electronic media. But, is it true that we just left the "pre-nano dark ages" ? Of course not! Nanoparticles and nanomaterials have been engineered/manufactured and used commercially since well before the above cited timeline. And the earliest large scale industrial applications involving natural nanomaterials are certainly more than 100 years old.

Instead of "nano", terms to describe these materials included (and still include): sub-micron, polycation, colloids, oligomers... and some chemical species that fit in today's definition of a nanoparticle were even considered as dissolved 20 years ago. A major difference between back then and today is the fact that, now, there is a special focus on hunting down any unique properties that are specifically linked to the nano-size of the material. Does this mean that we might have missed something important for all those nanomaterials from pre-nano age ? It is, of course, almost impossible to tell. What might be instructive however is to re-examine what we learned about the unusual properties and/or reactivity of these "old" compounds in terms of modern nano-science.

What did we find out back then, what are we doing different now ? Examples taken from the chemistry of rather common elements in the environment, viz. aluminum, iron and silicon and some combinations thereof, will be re-analyzed in terms of what was called "unusual" or "surprising" behavior, and just might have been the first hints of the nano-specific properties we are focussing on today. Remembering the reactivity and toxicity of "historical nanoparticles", like the somewhat elusive Al<sub>13</sub> species (and its Fe counter part), will naturally lead to comparisons to our more recent studies addressing the properties of the same type of nanoparticles, i.e. various types of Fe oxides and natural nanosized aluminosilicates.

# Using Predictive Toxicological Paradigm to Study Nanotoxicity and Design Safe Nanomaterial

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With the rapid implementation of nanotechnology and the accompanying rate by which commercially available nanomaterials are being introduced, we need a blueprint for safe design of nanomaterials. We advocate a predictive toxicological paradigm for the assessment of nanomaterial hazard and safe design. The predictive toxicological approach is defined as establishing and using mechanisms and pathways of injury at a cellular and molecular level to prioritize screening for adverse biological effects and health outcomes *in vivo*. Specifically as it relates to nanomaterials, a predictive approach has to consider the physicochemical properties of the materials that could lead to molecular or cellular injury and also has to be valid for predicting the possibility of adverse health outcomes in humans and impact organisms. The hierarchical oxidative stress paradigm (HOP) is an example of an injury mechanism that can be used to explore the potential that some types of nanomaterials may generate oxidant injury at intact animal level. As an example, among three metal oxides (ZnO, CeO<sub>2</sub> and TiO<sub>2</sub>), ZnO was clearly the most toxic in HOP screening in bronchial epithelial and macrophages cell lines. This toxicity is premised on particle dissolution and shedding of toxic Zn<sup>2+</sup> that is responsible for cellular ROS production and pro-inflammatory responses. Another example is cationic nanoparticles, including amino-polystyrene and polyethylenimine (PEI)-coated mesoporous silica nanoparticles (MSNP). These particles induce cationic toxicity that is premised on high cellular uptake leading to lysosomal and mitochondrial injury, including cellular ROS production. In addition to serving as a useful test platform for the assessment of nanomaterial hazard, the HOP may be also be of considerable importance for the safe design of nanomaterials. Thus, changing the dilution chemistry of ZnO and cationic density of PEI-coated MSNP can be shown to reduce their toxicity as reflected by HOP screening. This constitutes an ideal nano-EHS paradigm that utilizes quantitative structure-activity relationships for the safe design of nanomaterials that have biological and commercial applications. Examples are decreasing ZnO nanoparticle dissolution by iron doping and attachment of shorter length PEI polymers to the MSNP surface. ZnO nanoparticles are used in sunscreens while PEI coating is useful for the delivery of siRNA and plasmid DNA.

# CdSe Quantum Dot Intracellular Fate During Trophic Bioaccumulation in Protozoa as Assessed by STEM/EDS

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Microorganisms are the lowest trophic level of ecosystems, and when engineered nanomaterials (ENMs) are present, bioaccumulation and bacterivory could lead to biomagnification in higher trophic levels. This research aims to understand the type, degree, and location of QD bioprocessing during trophic transfer, including the use of quantitative STEM/EDS approaches in ENM fate research. Previous work showed that CdSe Quantum Dots (QDs) limited the growth of *Pseudomonas aeruginosa* (PA) and caused elevated levels of reactive oxygen species; scanning transmission electron microscopy (STEM) and energy dispersive spectroscopy (EDS) revealed extensive membrane damage and intracellular QDs<sup>(1)</sup>. The goal of the work reported here was to understand how QD- and Cd(II)-fed PA impacts growth of the ciliated protozoan *Tetrahymena thermophila* in a simulated freshwater environment, how QDs and Cd(II) bioaccumulate in *Tetrahymena*, and the associations of QDs and Cd(II) with cellular structures. In a related presentation (Werlin et al.) we demonstrate the trophic transfer of QDs from PA to *Tetrahymena* cells, with accompanying toxicity and extensive biomagnification. In this presentation we report the distribution of QDs in *Tetrahymena* cellular structure.

Bacteria, PA, were grown with and without either QDs or Cd(II), washed free of these reagents and were then fed to *Tetrahymena* cells. Accumulations were visualized by STEM/EDS over a 24 hr feeding period. STEM images showed the presence of QDs within *Tetrahymena* at 4 hours; by 24 hours the quantity of QDs had increased but remained unequally distributed within the protozoan cells. Unexpectedly, the highest density of QDs appeared to occur in mitochondria, which could have accelerated *Tetrahymena* death. EDS is being used to assess the co-localization of QD-associated Cd and Se in the *Tetrahymena* cells, and to further evaluate, through comparing the atomic ratios of these elements, QD integrity in different cellular structures.

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# **Analysis of Nanoparticle Toxicity using Machine Learning Techniques**

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The main aim of the study is to investigate the application of machine learning techniques to the analysis of the toxicity of nanoparticles. In the first phase of the study the toxic properties of eight different nanoparticles, namely, silver, gold, platinum, aluminium oxide, iron oxide, silicon oxide, cadmium-telluride and zinc oxide is being evaluated. The test data consists of five different cytotoxicity parameters measured for ten incremental doses and eight incremental duration of exposure of the nanoparticles under study. In order to evaluate the relevance and dependency of the toxicity parameters, a classification approach has been adopted utilizing kernel algorithms. The goal of the study is to develop a classifier for nanoparticle toxicity that will enable mapping of nanoparticle physicochemical parameters with the observed toxicity measures.

# A thermophoretic sampler for collecting airborne nanoparticles

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Airborne nanoparticles pose a serious risk to the human respiratory and cardiovascular systems due to their small size and high surface-to-volume ratio (1, 2). Detailed characterization of such particles is just beginning because existing aerosol methods are more conducive for larger particles; new techniques are needed to describe particles in nanoscale detail. Transmission electron microscopy (TEM) has been growing in popularity for characterizing airborne particles, but collecting samples for TEM can be challenging because the optimal amount of particles for imaging must be isolated, and the sampling method can introduce bias in particle size. The objective of this work was to design and test a thermophoretic sampler for the predictable collection of airborne nanoparticles onto TEM grids using low temperature gradients.

A variety of techniques have been used to collect particles on TEM grids; impaction and placement of grids on filters are better suited for larger particles. Among published work, thermophoretic deposition is a popular sampling technique for collecting nanoparticles on microscopy grids (3-6). The thermophoresis phenomenon is observed when a high temperature gradient is created over a small distance in a gas. Due to the dependence of Brownian motion on temperature, ultrafine and nanoparticles migrate from the hot region to the cold region (7). This technique allows for viewing the morphology and size of nanoparticles and aggregates in almost undisturbed conditions and selectively collects nanoscale particles, since larger particles have much lower Brownian diffusivities (3).

We have designed a low-cost thermophoretic precipitator that uses ice water as the cooling source and a 10-W resistor as the heating source. The deposition area was designed to be small (15 mm × 4 mm), so that deposition is focused on the 3-mm TEM grids. Up to three grids may be aligned along the deposition area. The thermophoretic chamber's height can be adjusted by mounting Teflon sheet separators of different thicknesses; 1 mm was used for this study. The temperature gradient was  $136 \pm 7 \text{ K cm}^{-1}$  (standard deviation), and the flow rate was  $6.0 \pm 2.0 \text{ mL min}^{-1}$ .

The precipitator was evaluated by flowing synthetic aerosols through it and visualizing the samples on a TEM (Philips, model EM420). An atomizer (TSI, model 3076) with diffusion dryer and neutralizer was used to aerosolize silver nanoparticles (AgNPs) and 80-nm polystyrene latex spheres (PSL80s). Both test aerosols were characterized in terms of particle size distribution and concentration using a scanning mobility particle sizer (SMPS) spectrometer (TSI, model 3936). The total particle concentrations were  $8.04 \times 10^4 \text{ particles cm}^{-3}$  for AgNP + EDTA (ethylenediaminetetraacetic acid used as a stabilizer), with a median diameter of 20 nm, and  $2.22 \times 10^5 \text{ particles cm}^{-3}$  for the PSL80 aerosol. Bright field, dark field, and diffraction pattern images were obtained.

In both cases, one grid was collected prior to applying a temperature gradient. Then, two grids (Ted Pella, Carbon Type-B 300 mesh) were inserted in the precipitator to collect particles with a  $136 \text{ K cm}^{-1}$  temperature gradient. Electron microscopy images confirm that particles were successfully collected in the grids subjected to a temperature gradient. A very small particle count was observed on the grids that were not subjected to a temperature gradient (zero to 1 particle per grid hole). About 0-3 particles were observed per grid hole for the AgNP samples, which were collected over a period of 1 hour. Conversely, a very large number (i.e. >500) of particles were visualized per grid hole for the PSL80 samples, which were collected over a period of 2 hours.

An amorphous matrix was observed around and over particles, probably originating from the stabilizers used in both nanoparticle dispersions, i.e. EDTA, in the case of the AgNPs, and surfactant in the case of the PLS80s. Small (<10 nm) particles were observed in both cases. Spherical non-crystalline 80-nm particles were observed in the PSL80 grids, and crystalline particles measuring approximately 30 nm were observed in the AgNP samples. Preliminary analysis of diffraction patterns confirmed that the observed particles are indeed made of silver. There were also crystalline nanosized sheets observed in both PSL80 and AgNP samples, which were probably formed from aerosolization and drying of impurities present in the solutions. Preliminary results show that this precipitator was effective in collecting nanoparticles of a wide size range and will be effective for use in future studies of airborne nanoparticles.

### **Acknowledgements**

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# **XAS Investigation of the Fate of Fe<sup>0</sup> Nanoparticles in the Environment and Implications for Exposure and Toxicity**

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The reactivity toward target contaminants, fate, transport, and toxicity of iron nanoparticles has been shown to be dependent on the types of mineral species present within the particle<sup>1-5</sup>. Nanoscale zero-valent iron is an engineered nanomaterial that is intended for in situ groundwater remediation and initially contains a very high reducing potential<sup>6</sup>. Thus it reacts very quickly with many ubiquitous geochemical constituents, including water, to oxidize its surface. In this study it is shown that the short-term kinetics of particle oxidation are anion dependent, and so are the types of oxidized iron species formed. In addition, the profound effect of dissolved oxygen on the speciation of the oxide formed and oxidation kinetics is discussed, and the passivating effect of nitrate at various concentrations is reported. Each of the findings here will be placed into context of eventual reactivity, fate, transport, and toxicity of NZVI that has been exposed to the environment.

NZVI particles were aged in the presence of common groundwater anions and excess dissolved oxygen, for short-term and longer-term (6 months) periods of time. Characterization of the oxidation state and identification and quantization of the mineral species formed was performed using X-ray absorption spectroscopy (XAS) at the Stanford Synchrotron Radiation Laboratory (SSRL), at the Fe K-edge (7112 eV). X-ray near edge structure (XANES) comparison between particles aged in the presence of different groundwater anions revealed the relative extent of oxidation. The extended X-ray absorption fine structure (EXAFS) region of the XAS spectra was fit using an iron mineral model compound library that had been compiled; a method employing principle component analysis (PCA) and linear combination fitting (LCF) was used to identify and quantify the mineral species that had formed.

## **Acknowledgements**

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scientists at SSRL, and Lee Penn and Brady Forsberg at the University of Minnesota Department of Chemistry for model compound synthesis.

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# **UC CEIN Protocols Project: Tools and Guidelines for Development and Validation of Standard Protocols**

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A critical goal of the UC CEIN is to develop standard protocols for studying the environmental implications of nanotechnology that are used across the center and can be disseminated to external stakeholders. This standardization is essential not only to our efforts to integrate data from different sources and laboratories within the UC CEIN but also to international efforts to produce a set of robust assays for studying the fate and transport of nanomaterials in the environment and their biological and ecological impacts. Here, we present guidelines and tools for the development and validation of standard protocols that are being used in the UC CEIN.

# UCLA Molecular Shared Screening Resource: Capabilities and Applications for Nanotoxicity Studies

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The central goal of the UC Center for Environmental Implications of Nanotechnology (UC CEIN) is to develop a broad-based model of predictive ecotoxicology of nanomaterials (NM) premised on quantitative structure–activity relationships (QSARs) and NM injury mechanisms at the biological level. Establishing a predictive science is a timely approach for nanotechnology-based enterprises wishing to avoid the problems faced by the chemical industry, where only a few hundred of the *ca.* 40,000 industrial chemicals have undergone toxicity testing, making it very challenging to control the toxicological impact of chemicals in the environment. A recent report by the National Research Council of the National Academy of Sciences set forth a vision of dramatic change in toxicological testing from individual testing to a predictive high-throughput paradigm premised on the established mechanisms and pathways of toxicity. Within the UC CEIN, we are using high throughput screening (HTS) to develop a rich database of toxicological outcomes for nanomaterials that provides the large volumes of data needed to develop a robust predictive model and also allows us to prioritize which materials should be the focus of more in-depth low-throughput studies at the organismal and population level.

The hallmarks of HTS are high sample throughput, miniaturization, and full automation of the assay process. This approach requires robotics, data processing and control software, liquid handling devices, and sensitive detectors. Although developed in the pharmaceutical industry, HTS is not limited to drug discovery: it can be used to quickly conduct thousands of tests for any assay that is amenable to automation. We are using existing HTS capabilities at the UCLA Molecular Screening Shared Resource (MSSR) to rapidly determine the specific features of NMs that govern their biological interfacial properties, environmental fate, and transport.

Established in 2003, the MSSR provides HTS technology. Since 2005, it has been

directed by Ken Bradley and Robert Damoiseaux, who report to an Advisory Board with members from UCLA's CNSI, Chemistry, Biology, Medicine, and other departments. The MSSR contains two fully integrated systems: (i) Automated liquid handling, multiple plate reading, plate filling and washing, deshiielding, and delidding, and online incubators for cell-based assays using a Beckman/Sagian system equipped with an Orca robotic arm that delivers plates to individual work stations. Current capacity of cell-based assay is ca.  $10^5$  wells (conditions)/day. Multiple plate readers allow fluorescence, FRET, BRET, time-resolved fluorescence, fluorescence polarization, luminescence, and UV-Vis absorption assays. (ii) A second Beckman/Sagian Core system for HCS using automated microscopy with an Orca arm and a Molecular Devices ImageXpress (micro) automated fluorescence microscope and a Cytomat 6001 incubator. Additional equipment in the MSSR that is available for off-line use includes a Genetix Q-bot colony-picking robot to maintain and re-order clone collections; a Hydra 96 and Precision 2000 automated pipetting and manifold; 6-ft cell culture facilities, molecular biology capabilities,. MSSR screening capabilities include two genome-wide knockout libraries of *S. cerevisiae* yeast, a genome-wide knockout library of *E. coli*, and genome-wide small interfering RNA libraries for mouse and human, providing functional genomic capabilities for identifying cellular pathways governing responses to nanomaterials. Here, we discuss how these robotic systems and libraries can be used for toxicological screening, using examples of systems being studied in the UC CEIN.

# **Bacteria-nanoparticle interactions: insights from axenic cultures**

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Bacteria are integral to natural ecosystems where their growth facilitates biogeochemical cycling and bioremediation. Any perturbation, such as the introduction of contaminants, could alter bacterial survival and growth, and thus ultimately effect ecosystem function. Here, we focused on manufactured nanomaterials (MNMs) as environmental contaminants and studied the potential effects of MNMs (such as ZnO and TiO<sub>2</sub>) using model bacteria in laboratory culture: *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. Two types of liquid culture chemistries were tested: complex, rich media (Luria Bertani broth), and several minimal media formulations. The latter were selected to recreate oligotrophic conditions expected in nature, to facilitate MNM dispersion, and to avoid extensive MNM dissolution by chelators. Based on bacterial growth patterns in media amended with different concentrations of either ZnO or TiO<sub>2</sub>, dose-response relationships were established for the three different bacterial strains. In addition, environmental scanning electron microscopy revealed a differential association of MNMs between Gram negative and Gram positive bacteria. Overall, our preliminary experiments have identified a culture medium for studying environmentally-relevant effects of MNMs on bacteria and provide potentially useful information to plan and conduct our future experiments, which will focus on understanding bacterial responses to MNMs, variations across strains, the mechanisms by which MNMs reduce bacterial growth, and the fates of MNMs in the presence of bacteria.

# Gene Expression of *P. aeruginosa* exposed to Cd(II) salts and CdSe Quantum Dots

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There is an urgent need for fundamental research regarding mechanisms of engineered nanoparticles (ENPs) toxicity at the cellular level, both for developing transferable paradigms in nanotoxicology, and also to provide sensitive tools for monitoring the presence and effects of ENPs in environmental compartments. This research will investigate the genome-wide gene expression changes in *P. aeruginosa* in response to Cd(II) salts and cadmium selenide quantum dot (CdSe QD) exposure in order to compare the genetic response to Cd(II) salts versus cadmium nanoparticles.

In a recent study, Priester *et al* (1) showed that, on a dissolved cadmium basis, CdSe QDs inhibited growth of *P. aeruginosa* comparably to Cd (II) up to a cadmium concentration threshold. Above that concentration, QDs were significantly more inhibitory. These findings raise many questions regarding whether QDs and Cd(II) act through similar toxicity mechanisms at low concentrations, and what accounts for the apparent “nanoparticle effect” at higher concentrations. In analyzing the gene response of *P. aeruginosa* to Cd(II) and CdSe QDs, particular attention will be given to genes encoding crucial enzymes involved in reactive oxygen species (ROS) elimination, peroxidase and superoxide dismutase, as well as Cd(II) export proteins implicated in cadmium resistance.

## Acknowledgements

This project received support from the University of California Toxic Substances Research & Teaching Program <http://tsrtp.ucdavis.edu>.

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## **In vitro toxicity study of PLGA and HA nanoparticles**

Sijing XIONG<sup>1</sup>, Xinxin ZHAO<sup>1</sup>, Boon Chin HENG<sup>1</sup>,  
Joachim Say-Chye Loo<sup>1,2</sup>, Kee Woei NG<sup>1</sup>

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Poly-(D,L-lactide-co-glycolide) (PLGA) and hydroxyapatite (HA) has been widely studied for drug delivery, because they are biocompatible and biodegradable. However, the toxicity of PLGA and HA nanoparticles has not been adequately investigated. The aim of our research is to determine how the different physiochemical properties can influence the biocompatibility and toxicity of PLGA and HA nanoparticles. We successfully synthesized spherical PLGA nanoparticles with average size of 70nm, 100nm, 130 nm, 200 nm, 250 nm, 300nm and nano-sized, rod like HA crystal with the average length of 60 nm, 120 nm and 240nm. The biocompatibility of PLGA and HA nanoparticles was determined by using human bronchial epithelial cell line (BEAS-2B) and Human hepatocellular liver carcinoma cell line (HepG2) and murine monocyte-derived macrophage (RAW264.7) cell models. The metabolic activity of cells was evaluated by WST-8 assay. In Vitro exposure of three cell lines to both PLGA and HA nanoparticles within the range of 10-300 ug/ml did not significantly alter cell viability. The inflammation of cells after treated by HA nanoparticles was evaluated by DHF assay which revealed that BEAS-2B generated more reactive oxygen species (ROS) than RAW264.7 and HepG2 (BEAS-2B>RAW264.7 > HepG2) and longest rod HA230 generate highest level of ROS (nHA240>nHA120>nHA60).

# **Health Effects Risk Assessment of Nominated Nanomaterials and Trends in the Nanotoxicology Literature**

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Environmental Health Sciences, Molecular Toxicology IDP, University of California, Los Angeles, CA 90095, USA

The University of California Lead Campus for Nanotoxicology Research and Training Program has nominated a list of 9 nanomaterials to be prioritized for study:

1. Carbon Black
2. Carbon Nanotubes
3. Ceria
4. Den-drimers
5. Fullerenes
6. Quantum Dots
7. Silica
8. Titania
9. Zinc Oxide

It is important to know progress that has been made on each so future research can be directed towards areas where information is lacking. In addition, the emerging industry of manufactured nanomaterials will create many more nanomaterials that need to be studied. Identifying similarities between the nominated nanomaterials can guide toxicology research for these new nanomaterials.

An extensive literature review was conducted for each nanomaterial. Within the past year, the amount of literature addressing the health effects of these nanomaterials has grown significantly. However, some nanomaterials have been studied more than others. For example, titania and carbon nanotubes have an abundance of literature, whereas ceria and den-drimers lack studies and toxicological data.

A risk assessment has been written for each of the 9 nanomaterials. In addition, a chart comparing each of the nanomaterials to each other is published on a website ([www.ph.ucla.edu/ehs/tsrtpnano](http://www.ph.ucla.edu/ehs/tsrtpnano)). The website includes summaries of all reviewed journal articles, links to primary literature, downloadable EndNote® database, and an overview of the patterns between the nanomaterials. The website was constructed to aid the nanotoxicology research community in finding current literature and quickly assessing where the gaps in knowledge are for the nominated nanomaterials.

# ***In vivo* Evaluation of Multi-walled Carbon Nanotubes Toxicity to Aquatic Organisms**

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Carbon nanotubes (CNTs) are one of the most attractive nanomaterials and have increasingly been used in commercial products because of their unique properties. It is therefore no surprise that the increase of CNTs in production and applications will lead to great risks of human and environment exposure to these nanomaterials (1-3). Hence, it is necessary to evaluate the toxicity of CNTs to living organisms before their large-scale manufacture. The present study aims at investigating the potential impact of CNTs on three different trophic-level organisms in freshwater ecology, that is, luminescent bacterium Q67, *Daphnia magna*, and Japanese mekada (*Oryzias latipes*) embryos.

Multi-walled Carbon Nanotubes (MWCNTs) were used as obtained from Ningbo Huashi Nanotechnologies, Inc. The purity and diameter of MWCNTs provided by the manufacture were above 97.89% and ranged from 20-40 nm, respectively. XPS, FTIR, and Raman spectra indicated that oxygen-contained groups and disordered carbon were present in the MWCNTs. Figure 1A shows the acute toxicity of MWCNTs to luminescent bacterium Q67. Below the concentration of 20 mg/L, MWCNTs did not have obviously toxicity of Q67, and even when the concentration was up to 100 mg/L, the luminescent inhibition was about 30%. These data indicated that MWCNTs had no strong toxicity to Q67. Also, no acute toxicity of *D. magna* to MWCNTs was observed. However, there was significantly chronic reproduction toxicity of *D. magna* to MWCNTs even at the concentration of 0.5 mg/L during 14-day exposure (Figure 1B). During exposure, the control group had 86.29±9.55 offspring, while there were only 44.17±23.78, 16±9.36, and 26.28±19.68 offspring, at the concentration 0.5, 2, and 5 mg/L, respectively. Therefore, it is necessary to evaluate the chronic toxicity of nanomaterials to organisms.

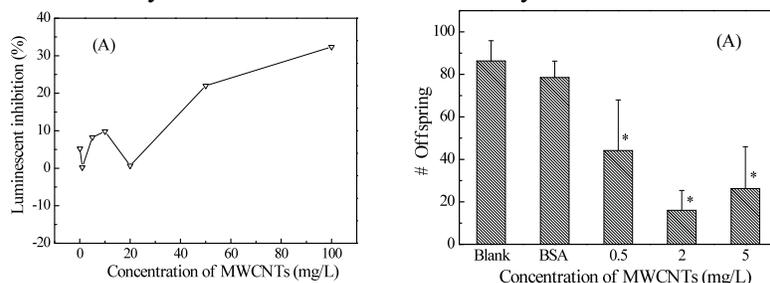


Figure 1. (A) Inhibition efficiency of MWCNTs to luminescent bacterium Q67; (B) Offsprings of *D. magna* after 14-day exposure MWCNTs ( $p < 0.05$ ).

During 14-day exposure, it was observed that *D. magna* could adsorb or uptake MWCNTs into/on their body. Also, we observed that the dead *D. magna* after producing

offspring was usually with much MWCNTs on its body and the death occurred just before it molt. However, it was difficult to draw a conclusion that the adsorbed MWCNTs would inevitably induce the death of *D. magna*. Interestingly, molting was one of the manners for *D. magna* to remove MWCNTs out of its body (Figure 2). Scanning electron microscopy confirmed these black matters had tube structure as CNTs.



Figure 2. Photos of *D. magna* exposure to MWCNTs in different time.

MWCNTs had significant toxicity to the development of Japanese mekada embryos with dose-effect relationship (Table 1). The hatching efficiency was below 50% when the concentration was 20 mg/L, and the hatching time was also delay 1.5 d. As MWCNTs could not penetration into the chorion of embryos (4), disturbance of the composition of the culture or the properties of chorion might be the toxicity mechanisms of MWCNTs to embryos.

Table 1. Effect of MWCNTs on Japanese mekada embryos hatching

Concentration (mg/L)	Hatching efficiency (%)	Hatching time (d)	Deformity (%)
0	85.0±7.0	9.0±0.3	2.5±3.5
2	77.5±24.7	9.6±0.7	2.5±3.5
8	58.3±15.3	9.2±0.5	1.7±2.9
20	48.3±20.8	10.5±0.5	0

Therefore, MWCNTs had chronic toxicity to aquatic organisms although obvious acute toxicity was not observed. The chronic toxicity effect could be displayed on the population-level.

#### ACKNOWLEDGEMENTS

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# **Aggregation of ZnO nanoparticles under different aqueous solution chemistries**

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The aggregation of ZnO nanoparticles influences not only their environmental transport but also their toxicity. In natural aquatic systems, the ubiquitous presence of natural organic matter (NOM) can lead to interactions with released ZnO nanoparticles (NPs) and influence their transport. In this study, the aggregation behavior of ZnO with and without NOM under different ionic strength and initial pH were examined by both dynamic light scattering and sedimentation techniques. ZnO aggregates faster as the pH approaches its point of zero charge. An higher initial ZnO concentration leads to faster aggregation. The role of ionic strength was examined by determining the reaction-limited cluster regime (RLCR) and diffusion-limited cluster regime (DLCR). We employed DLVO calculations to explain these observations. In the presence of NOM, ZnO aggregation was significantly slowed. The final aggregate size increased with an increase in NOM concentration, which may be explained by the bridging effect as well as an increase in the negative surface charge of ZnO. Finally, the fractal dimensions of ZnO aggregates under different conditions were determined by static light scattering (SLS).

## Education & Outreach Activities in CEINs

Thursday, September 10, 2009

2:00 p.m. – 3:00 p.m.

Auditorium 151/155

# **Engaging and Effectively Communicating our Results to Broad Range of Stakeholders: Education and Outreach Activities in the UC CEIN**

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The goals of the Education and Outreach initiatives within the UC CEIN include both activities to help integrate across the center and insure a unified and effective approach to mentoring and training students and postdoctoral fellows and activities focused on communicating the findings of the UC CEIN to a broader audience and varied constituencies and helping to frame these findings to provide a scientific basis for related policy and regulatory decisions. The objectives of the Education and Outreach initiatives are to:

- Train a diverse cohort of new scientists who are broadly trained to handle complex issues related to nanomaterials in the environment
- Train researchers to use appropriate safeguards when handling or disposing of nanomaterials
- Build a cohesive network of stakeholders with interests at the interface of nanoscience and the environment
- Maximize productive use of nanomaterials while minimizing environmental hazards
- Accurately communicate to the public the implications of nanotechnology in the environment

Towards this end, we engaged in the following activities in the first year of the center:

- A capstone course on Nanotoxicology was taught in the Department of Environmental Health Sciences at UCLA in Fall 2009 and made available via

webcast to students and postdoctoral fellows across the UC CEIN. In addition, through an arranged partnership, students at UCSB were able to take this course for credit through their own institution. A core class on Fundamentals of Toxicology which was offered in the Department of Environmental Health Sciences at UCLA in Spring 2009 was also made available via webcast to students and postdoctoral fellows across the UC CEIN. In addition, through an arranged partnership, students at UCSB were able to take this course for credit through their own institution

- A course on Nanotechnology and the Environment (Hoek) was taught in the Department of Civil Engineering at UCLA in Spring 2009 and made available via webcast to students and postdoctoral fellows across the UC CEIN.
- A new Training Course on Safe Handling of NMs (Suarez and Godwin) was developed and is being formatted for online access. Over the next year, we plan to implement the module at UCLA and UCSB and to assess the effectiveness of the module in collaboration with social science researchers at UCSB and Northwestern.
- A Working Conference on Nanotech Regulatory Policy was co-organized and co-sponsored by the UC CEIN (Malloy and Godwin) on April 17, 2009. The papers presented at this conference are to be published in an upcoming special issue of the UCLA Law Review.
- Two Journalist–Scientist Communication Workshops were hosted at UCSB (Freudenberg) and made available to made available via webcast to students and postdoctoral fellows across the UC CEIN.

This presentation will cover the highlights of the activities from the last year and will provide an overview of ongoing projects and opportunities for researchers across both the UC CEIN and the CEIN to engage in Education & Outreach activities going forward.

# **Education and Outreach Initiatives in CEINT: Targeted Communication of Results Across Diversified Audiences**

G. Kelly <sup>1</sup>

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The mission of CEINT's Education and Outreach initiatives is to educate a diverse cadre of students and the general public regarding nanoscale science and engineering to lay the foundation for a workforce that will develop nanotechnology in a sustainable fashion within an interdisciplinary, global context. Our goal is to translate our research findings, with a particular focus on environmental processes, benefits, and impacts into the language of risk assessment and to provide guidance to a broad cross section of stakeholders regarding current and future concerns surrounding the environmental implications of nanotechnology.

CEINT's Education and Outreach objectives include:

- Implementation of courses and programs at the university level at each of our institutions
- Coordination and shared development of educational materials
- Implementation of laboratory rotations among CEINT partners to provide interdisciplinary and global experience
- Public engagement through NISE Net activities and broad dissemination of Nano2Earth curriculum and on campus student research experiences
- Engagement of targeted stakeholders including NGOs, industry, academia, and government in public forums, workshops, and briefings
- Creation of an engaged community of researchers, students, and other stakeholders at the intersection of nanoscience and environmental impacts with use of video conferencing, web-based tools and other elements of cyberinfrastructure
- Actively increase participation of women and minorities in nanotechnology research and education at each partner institution and extend the reach of CEINT to engage additional institutions with predominantly under-represented minorities

CEINT's educational activities feature a comprehensive approach to G5-postdoctoral training with educational programs that infuse our findings into rigorous training on our campuses and activities that extend into our local communities and the world at large. We communicate our discoveries and technological developments through tightly integrated programs at many levels of educational enterprises that include curriculum development, undergraduate/graduate fellowships, seminar series, brownbag colloquia meetings, lab rotations, international and service learning experiences, community outreach, REU experiences, and annual workshops.

Several successfully implemented first year educational and outreach activities include:

- Creation of 3 new courses- a core course on Environmental Nanotechnology taught in the Department of Civil and Environmental Engineering (CEE) at Duke with shared materials and parallel versions offered at CEINT partner universities; a new course on Aquatic Geochemistry offered in CEE at Duke with a parallel course on Environmental Geochemistry taught at Stanford University;
- Four modified courses across partner institutions to include Center related environmental nano-science content or methodology (Environmental Modeling: A Bayesian Perspective, Nicolas School of the Environment, Duke University; Fate, Transport and Physicochemical Processes of Organic Contaminants in Aquatic Systems at CMU; Fundamentals of Surface Science, Interface, and Nano Behavior at Virginia Tech University; and Solid State Chemistry, Department of Chemistry at Duke University);
- Successful recruitment of 59 university students and post-docs to participate in CEINT's educational and research programs (assistantships, internships, lab rotations, international and service learning experiences) with participant ratings between 70-88.9% on indicators of adequate support, positive supervisors' involvement, enhanced communication skills, beneficial exchange of ideas, enhanced lab skills, expanded network of professional contacts, and intent for future collaborations;
- Publication of 10 manuscripts in peer-reviewed journals as well as 5 book chapters;
- Sponsorship of 2 major international conferences on nano-science, one at Howard University Fall '08 and the other as part of the International Water Association Particle Separation Conference held at Duke University summer 2009;
- Sponsorship of 7 major seminars and 8 center-wide colloquia on environmental nano-science;
- Refinement of our high-school nano-science curriculum "Nano2Earth" for national dissemination and professional development for high-school teachers;
- Presentations by CEINT faculty and students at the NISE Net affiliated NanoDays 2009 at the NC Museum of Life and Science to expand public awareness in the area of environmental nano-science;
- Formed partnership with local and national high schools to sponsor summer research experiences in CEINT labs at Duke for 8 high school students in addition to 3 REU students;
- Developed partnership with faculty from North Carolina Central University (NCCU), an HBCU, centered around the theme of sustainable nanotechnology and materials science to engage under-represented youth to work at the intersection of nanotechnology, energy and the environment and put in place a formal long-term collaborative research and education collaborative to recruit and train students from under-represented minority groups; share resources; and feature joint research projects that capitalize on faculty expertise from both universities.

Additional highlights of CEINT's Education, Outreach and Assessment activities will be presented as well as plans underway for future developments and collaborative opportunities.